POTENTIAL ALTERNATIVE TRANSCRIPTS OF SEX DETERMINING GENE *SOFF* AND TRANSFORMING *ASPARAGUS OFFICINALIS* L. WITH *AGROBACTERIUM TUMEFACIENS*

by

DAKOTA ANDREW STARR

(Under the Direction of JAMES LEEBENS-MACK)

ABSTRACT

Suppressor of Female Function, SOFF, is a sex determining gene in garden asparagus, *Asparagus officinalis* L., though its molecular function is unknown. *SOFF* is a member of the Domain of Unknown Function family 247. Alignment of *SOFF* with a closely related paralog in *A. officinalis* indicates the possibility of two splice variants: one with a membrane-spanning domain and another with an extra exon relative to other *DUF247* genes. Through RNA-seq analysis of male and female flower buds collected at different developmental stages I could not unambiguously validate these two splice variants. Development of a transformation protocol would enable targeted knockouts of genes in the nonrecombining region of the Y chromosome, including *SOFF*. Using elite garden asparagus lines, *Agrobacterium* transformations were done using reporters RUBY and GFP. A single callus indicative of RUBY expression was recovered and has begun developing shoots.

INDEX WORDS: *Asparagus officinalis*, *Domain of Unknown Function family 247 (DUF247),* alternative transcript, RNA-seq, sex determination, transformation, GFP, RUBY, Agrobacterium

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CHAPTER 1

INVESTIGATING ALTERNATIVE TRANSCRIPTS OF Y-SPECIFIC SUPRESSOR OF FEMALE FUNCTION (*SOFF*) IN GARDEN ASPARAGUS (*ASPARAGUS OFFICINALIS* L.)

Introduction

The genus *Asparagus* originated in South Africa and its range expanded to Europe and Asia. The ancestral mating system is hermaphroditic and dioecy, the separation of male and female genotypes, evolved between three and four million years ago (Bentz et al. in prep, Norup et al., 2015). *Asparagus officinalis*, garden asparagus, is a perennial, dioecious crop that is widely grown across the United States, Europe, and Asia. Male plants are preferred in agricultural settings which do not waste the plants' resources on developing fruit and seed for the next generation. Fields with male and female plants will produce berries and the resulting seedlings will act like weeds competing for resources (Ide et al., 2019; Nikoloff & Falloon, 1990; Robbins, WW, Jones, 1925; Sneep, 1953).

The first widespread varieties of garden asparagus typically had a 1:1 male: female ratio (Flory, 1932; Robbins, WW, Jones, 1925). Since male plants produce larger yields even though the spears may have a smaller diameter, breeders began to focus on producing lines with a greater male to female ratio. Breeders identified naturally occurring andromonecious plants (producing male and hermaphroditic flowers) in their fields that produced seed with a higher frequency of male offspring. Some of the male plants derived from selfing of an andromonecious plant only produced male offspring.

These plants were later called “supermales” and were found to contain YY sex chromosomes instead of the typical, XY. To identify supermales, many test crosses had to be completed because the molecular mechanisms underlying sex determination in garden asparagus was not known (Franken, 1970; Nikoloff & Falloon, 1990; Rick & Hanna, 1943; Sneep, 1953).

Female asparagus plants are needed to produce hybrid seed. When crossed with YY ‘supermales’ the product is XY male seed. Andromonoecious XY genotypes produce male and hermaphroditic flowers and can also be selfed, though as described above all the seed would not be male (Franken, 1970; Ide et al., 2019; Nikoloff & Falloon, 1990; Rick & Hanna, 1943; Sneep, 1953).

The first of ten chromosomes that make up the garden asparagus genome (N = 10) contains the sex determining region (Harkess et al., 2017, 2020). The Y-specific sex-determining portion of chromosome 1 is about 1 Mb long and harbors seven full gene models within a non-recombining region, while the X-specific region is about 150 kb long. Two of the seven genes, *Suppressor of Female Function* (*SOFF*) and *defective in Tapetum Development and Function 1* (*TDF1*), are involved in sex determination (Figure 1). *SOFF* is a member of a Domain of Unknown Function gene family, *DUF247* (Pfam family 3140) and *TDF1* codes for a R2R3 MYB transcription factor involved in tapetum development (Harkess et al., 2017, 2020).

SOFF is one of two known *DUF247* genes that are involved in plant reproduction across the tree of life. The other *DUF247* gene characterized from perennial ryegrass is hypothesized to play a role in its self-incompatibility system (Manzanares et al., 2016). The mechanistic function of *SOFF* in sex determination is not known at this time

(Harkess et al., 2017, 2020). There is a paralog of *SOFF* located on a different chromosome in garden asparagus, chromosome 5. This paralog, *SOFF-Like*, resembles most *DUF247* genes in that it only has two exons. *SOFF* has a third exon with sequence that does not align with other *DUF247* genes. Alignments of exons 1 and 2 of *SOFF* and *SOFF-Like* are over 93% identical. The end of exon 2 of *SOFF-Like* aligns with sequence in intron 2 of *SOFF* with only seven base pair differences across 108 aligned bases (Figure 2). This overlapping region could be translated as an open reading frame as an alternative transcript derived from the Y-specific *SOFF* locus. The objectives of this chapter are to computationally assess the sequence in this region of overlap and use RNA-seq data to assess whether the *SOFF* locus is producing alternative transcripts that extend into the intron of the annotated *SOFF* gene model. I also assess differences in gene expression using RNA-seq data from male buds collected at three developmental stages: during initial suppression of pistil development (<1mm), when male pistil development completely ceases (1-2 mm) and after cessation of pistil development but just prior to flower opening and anther dehiscence (2-5 mm) (Caporali et al., 1994).

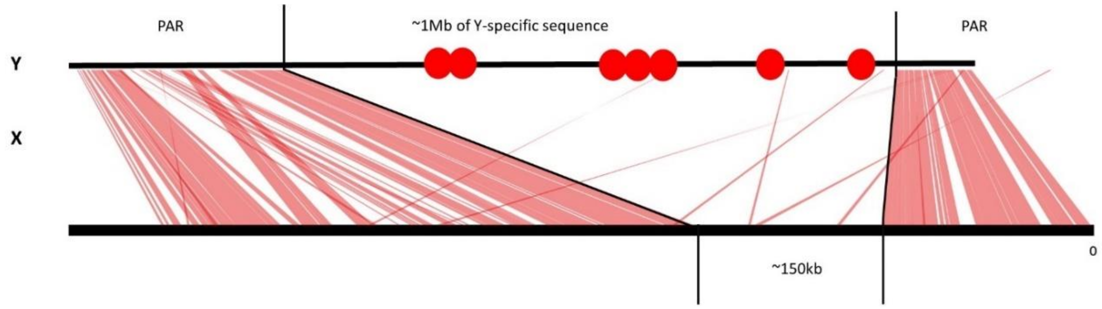
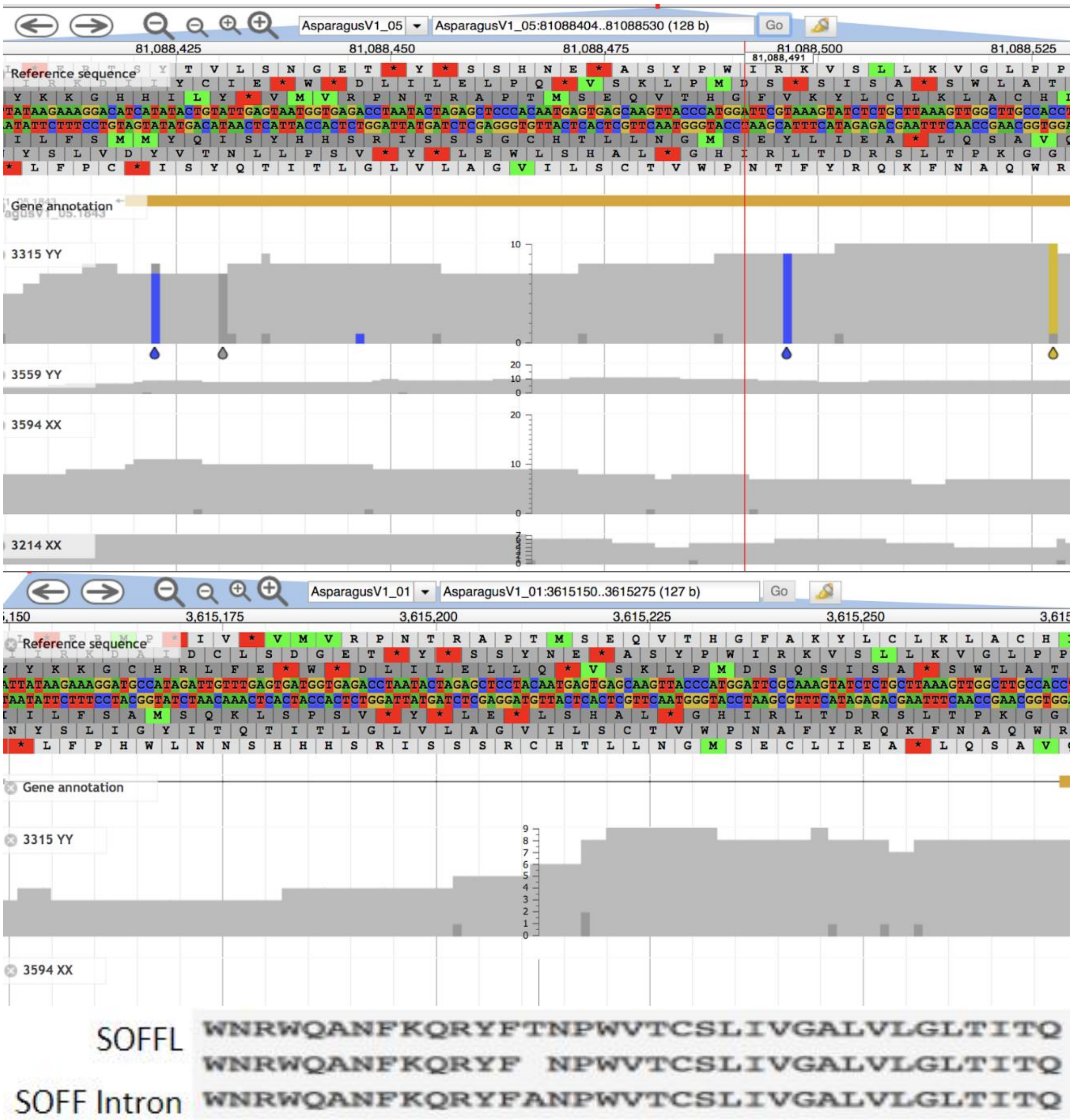


Figure 1. Garden asparagus gene models in the nonrecombining region. Figure adapted from Harkess et al. (2017) showing annotated gene models (red dots) in the nonrecombining region on the Y chromosome. PAR is the pseudoautosomal region.



SOFFL WNRWQANFKQRYFTNPWVTCSLIVGALVLGLTITQ
WNRWQANFKQRYF NPWVTCSLIVGALVLGLTITQ
SOFF Intron WNRWQANFKQRYFANPWVTCSLIVGALVLGLTITQ

Figure 2. Nucleotide and amino acid comparison of *SOFF-Like* and *SOFF.* Top image: *SOFF-Like* nucleotide sequence, translation in all 3 reading frames, and RNA-seq data for the end of exon two. Middle image: *SOFF* nucleotide sequence, translation in all 3 reading frames, and RNA-seq data for the beginning of intron two. Bottom image: comparison between the predicted amino acid sequence of *SOFF* extending into intron 2, and *SOFF-Like.* The only difference between the predicted amino acid sequences is an Alanine-to-Threonine substitution. *SOFF-Like* translation is right to left on the

bottommost reading frame. *SOFF* translation is right to left on the reading frame above the bottommost one.

Methods

Transmembrane helix prediction using TMHMM

To test whether there is a transmembrane domain in *SOFF*, the amino acid sequences for *SOFF*, the hypothesized alternative transcript of *SOFF*, and *SOFF-Like* were analyzed using TMHMM. TMHMM uses a hidden Markov model that predicts transmembrane protein topology to assess whether an amino acid sequence codes for transmembrane helix domains (Krogh et al., 2001; https://services.healthtech.dtu.dk/service.php?TMHMM-2.0). TMHMM produces a graph showing the probability that a transmembrane domain exists for the sequence provided.

Sampling and preparing garden asparagus buds for RNA-seq

Garden asparagus plants were grown in the botany greenhouses at the University of Georgia. Tissue from three male plants and one female plant were collected for sequencing. The male plants were from New Jersey stock (Jersey Giant or Jersey Knight) and the female genotype was F109 from a Californian breeding program.

To induce flowering, all growth was cut down to one inch above the soil. A few weeks after cutting back, the new spears produced flowers and were brought to the lab for sampling. Buds were collected in three different size classes based on length of the bud: ≤1 mm, 1-2 mm, and >2 mm to before flowering (3 mm for the female and 4-5 mm for males). The size for the buds were based on the staging work done by Caporali et al

1994. The development for the pistil (including carpels) in male flowers stops by the time the buds are 1.3 mm in length (Caporali et al., 1994). Buds were flash frozen in 1.5- or 2-mL tubes with liquid nitrogen. The buds were stored in a -80^{o}C freezer until RNA extraction. The buds were prepped for RNA extraction by adding liquid nitrogen to each tube and grinding the buds into a powder using a plastic pestle.

RNA was then extracted from the ground bud tissue using a Qiagen RNeasy Plant Mini Kit. The protocol for purification of total RNA from plant cells and tissues and filamentous fungi was used. 35 µL of RNAse-free water was added to the column to elute the RNA.

Samples were treated with Qiagen RNase-Free DNase using the DNase digestion of RNA protocol. DNA contamination and RNA concentrations were assessed on a Qubit using DNA and RNA standards, respectively. The RNA quantity and integrity was assessed on an RNA Bioanalyzer chip. All samples had at least 4.4 µg of high-quality RNA (RIN, RNA integrity number, values ranged between 5.8 and 9.3). Samples were stored at -20°C until they were sent on dry ice to HudsonAlpha Institute for Biotechnology (Huntsville, AL) for RNA-seq library preparation and 150 bp paired-end sequencing on an Illumina NovaSeq. We aimed to sequence ~30 million fragments per sample with paired ends. Samples include three genotypes as biological replicates for each of the male flower bud size classes. We had just a single female plant, so no replicates were collected for female bud size classes.

Analyzing RNA-seq results

RNA-seq samples were analyzed using the new Tuxedo suite (Pertea et al., 2016). HISAT2 was used to index the sequences to make them more efficient for read mapping, to map, and to produce sorted BAM files for each sample. StringTie was then used to assemble the reads for each gene and make a .gtf file for each sample. It also calculates the FPKM value (fragments per kilobase of exon model per million reads mapped) for differential expression analysis. The Ballgown R package was used to plot gene expression level distributions and compare female and male bud gene expression at size classes listed above (Pertea et al., 2015, 2016). Sorted BAM files were indexed using samtools and viewed in IGV (Integrative Genomics Viewer) version 2.12.3 to view reads that mapped to *SOFF* (Robinson et al., 2011). Pairwise comparisons of the three male flower bud size classes were performed to identify differentially expressed genes (DEGs) when comparing small and medium, small and large, and medium and large male flower buds.

Results/Discussion

Transmembrane helix prediction

Analysis of the amino acid sequence for *SOFF* did not reveal evidence for a transmembrane domain. The amino acid sequence of *SOFF-LIKE*, however, was predicted to include a transmembrane domain located in the last 25 amino acids of the C-terminus end (Figure 3).

The predicted amino acid sequence of *SOFF* exon two and intron two aligns well with that of the C-terminus end of *SOFF-Like* exon two. This may represent an alternative transcript of *SOFF* that has not been annotated in the garden asparagus genome. The predicted amino acid sequence for the open reading frame extending from

SOFF exon 2 into the second intron was analyzed to assess whether this hypothesized transcript may have a transmembrane helix domain (Figure 3).

The TMHMM data suggests that if the alternative transcript for *SOFF* exists then it should have the membrane spanning domain. If the alternative transcript is not present, then the mechanism that makes *SOFF* a sex determination gene may be the loss of a membrane spanning domain.

Comparison of gene expression in male and female buds

After receiving the RNA-seq data from HudsonAlpha, the reads were cleaned and mapped with HISAT2. Two of the samples had less than 30 million reads, Male1 large buds and the Female medium buds (see Appendix A Table 6 for sequence counts). Using the ballgown and ggplot2 R-packages a boxplot was made to assess the normalized distribution of FPKM values across the 12 libraries (9 male and 3 female, 1 for each bud subclass: small, medium, and large with three males sampled) (Figure 4). The cmdscale function was used to construct a multidimensional scaling (MDS) plot to assess Euclidean distances between transcript profiles (Figure 5). The Male2 small bud transcript profile was in a separate cluster away from the other samples. The Male large buds clustered in a group together. The rest of the samples were in a cluster together with the Female medium and large buds separated a short distance away. The Male2 small buds could have been developmentally behind the other small bud replicates.

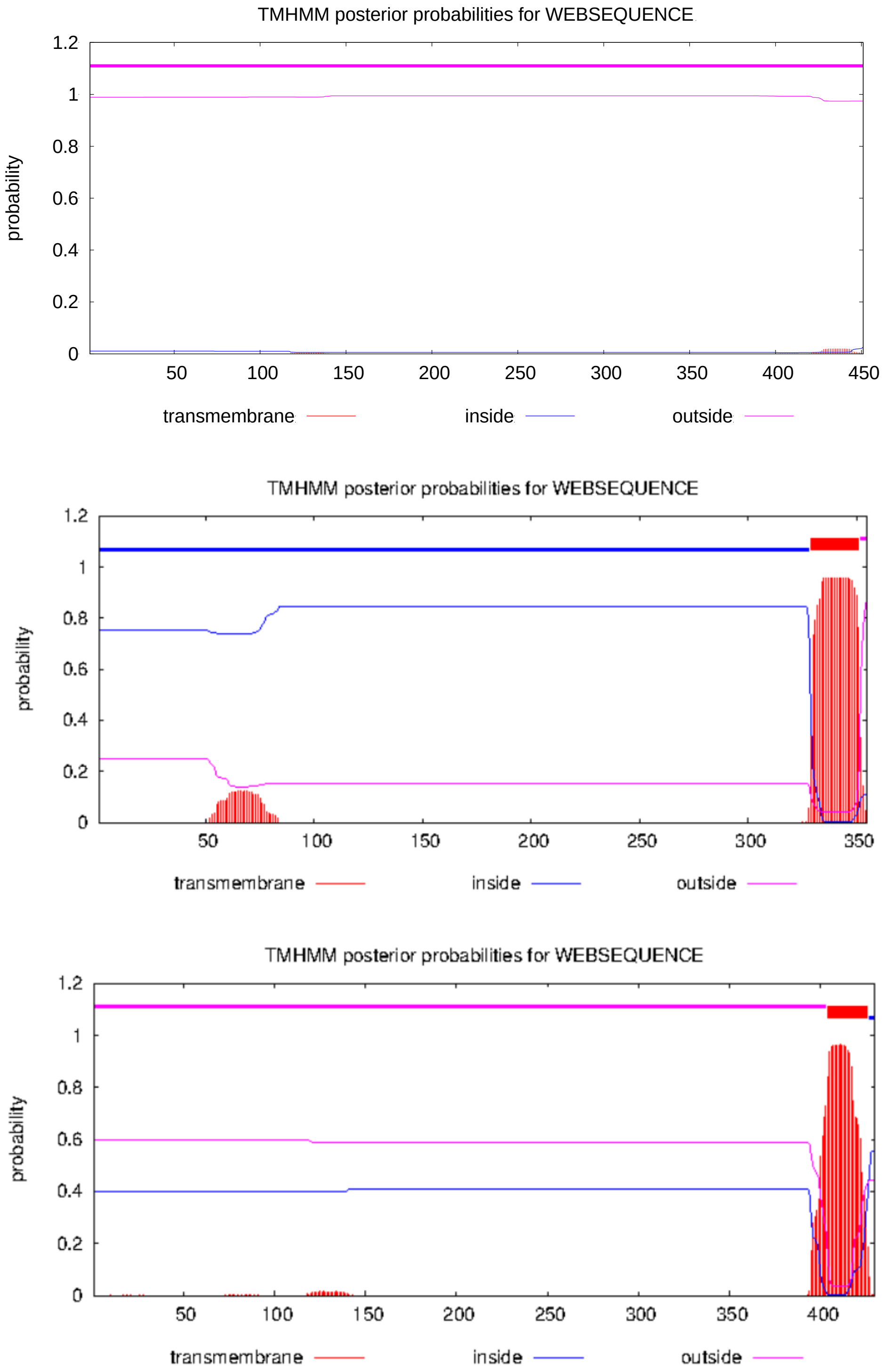


Figure 3. TMHMM output based on the coding amino acid sequence and probability of a transmembrane domain. The sequence for *SOFF* is the upper graph and *SOFF-Like* is the middle graph. The potential alternative transcript for *SOFF* is the lower graph.

Three pairwise comparisons of replicated Male flower bud size classes were performed to identify differentially expressed genes: i) small buds were compared to the medium buds, ii) small buds to large buds, and iii) medium buds to large buds. When looking at the differentially expressed genes between the small and medium buds there were 2029 with a p value ≤ 0.05 and the smallest false discovery rate (q value) for rejecting the null hypothesis of equal expression levels was 0.062. For the small and large bud comparison there were 5615 genes with a p value ≤ 0.05 and 838 of these genes had a q value ≤ 0.05. Two hundred and twenty five of the 838 genes mapped to gene models in the *A. officinalis* genome annotation (Harkess et al., 2017). For the 613 genes without a gene model listed, the merged StringTie gtf was greped for any gene models that overlapped at the 10% level. This produced a list of 420 genes that overlapped with at least one gene model. None of the 645 genes with a gene model, nor any of the remaining 193 putative gene transcripts inferred by StringTie with false discovery rates less than 0.05 mapped to the nonrecombining sex determination region (Appendix A Table 7 and Table 8). Between medium and large buds there were 2385 genes with a p value ≤ 0.05 but the smallest q value was 0.072.

The Integrative Genomics Viewer (IGV; Robinson et al., 2011) was used to view the RNA-seq reads and to assess RNA seq reads mapping to the *SOFF* gene region and assess whether the hypothesized alternative transcript for *SOFF* was being expressed. The female buds only had reads in the third exon (derived from paralogous autosomal regions) as expected from previous work. There were not enough reads to tell whether there was an alternative transcript being expressed in the male buds due to low expression or not enough sequencing depth (Appendix A Figures 9-12).

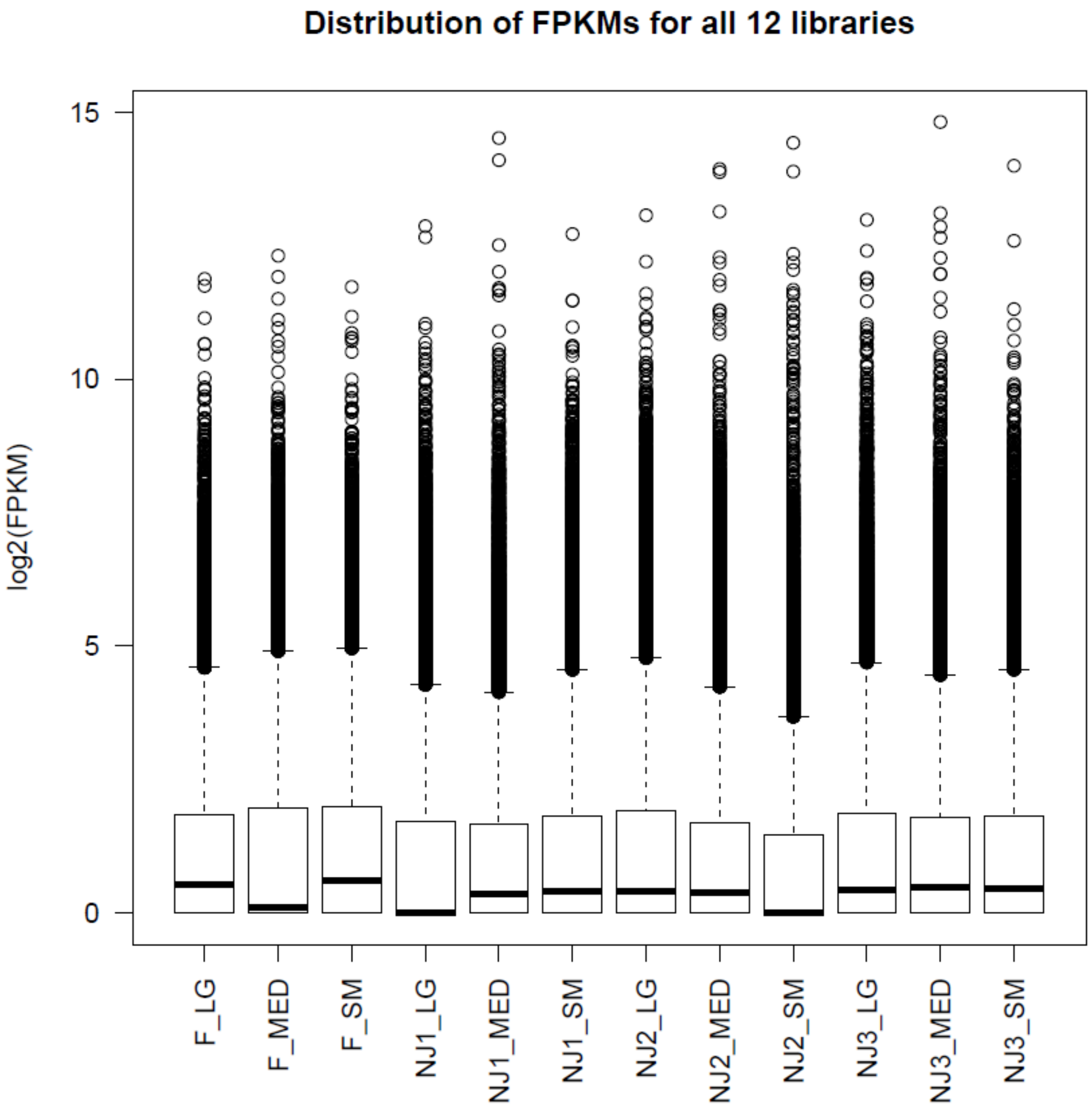


Figure 4. FPKM distribution for the 12 RNA-seq libraries. SM is small buds, MED is medium buds, and LG are large buds. NJ1, NJ2, and NJ3 are male buds and F is female buds. The reads were normalized to reduce bias from differing amounts of reads.

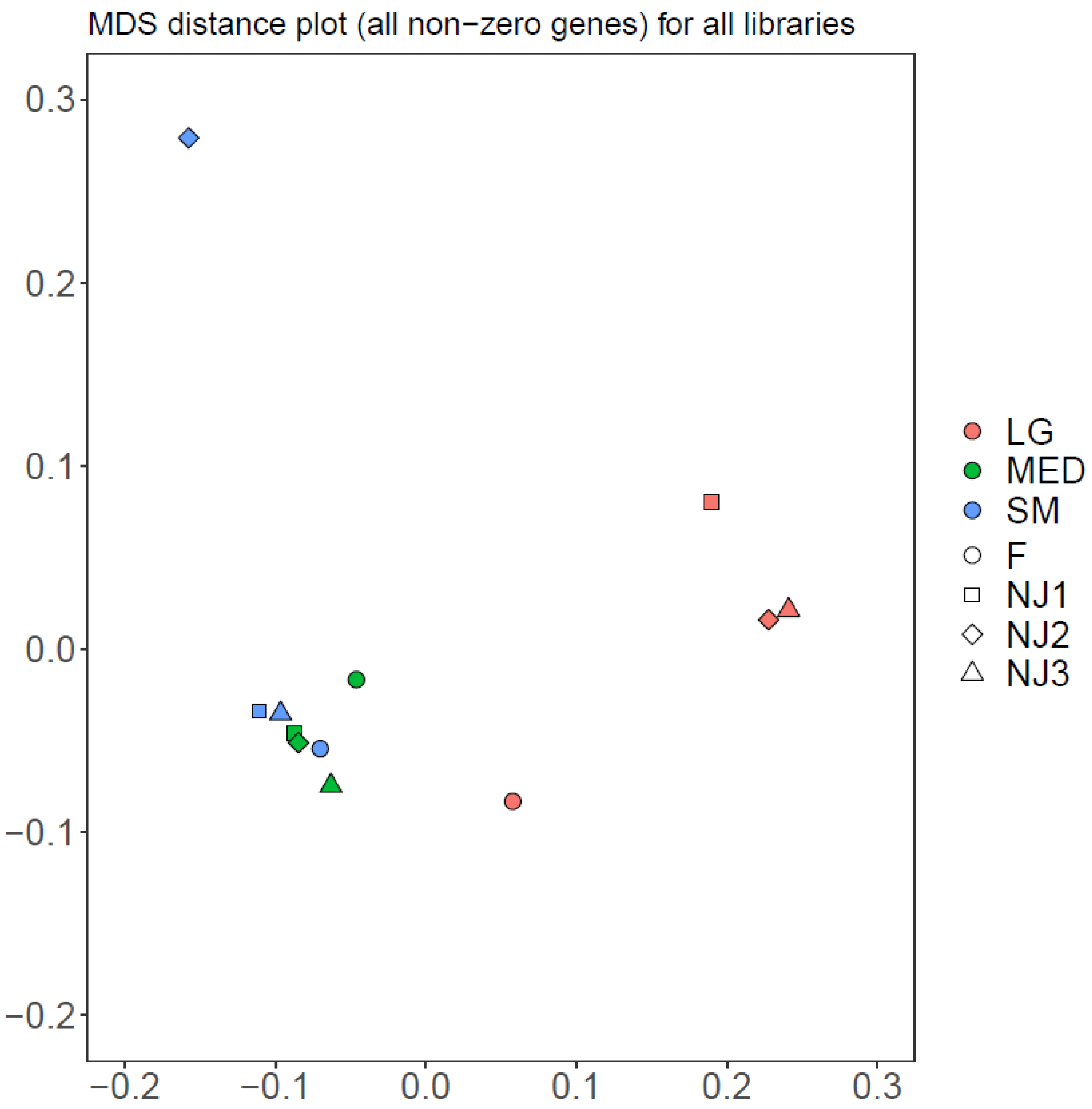


Figure 5. MDS plot of RNA-seq data. SM is small buds, MED is medium buds, and LG are large buds. NJ1, NJ2, and NJ3 are male buds and F is female buds.

Discussion and Future Directions

Pairwise analysis of the RNA-seq data from the male replicates only provided genes for the comparison between small and large buds for a q value of ≤ 0.05. This threshold value was chosen as an acceptable false discovery rate given a p-value of ≤ 0.05 for rejection of the null hypothesis (equal expression levels). The reason the small and large buds have significantly differentially expressed genes, and the other two comparisons do not is likely due to the fact that these buds represent more distinct developmental stages than the other two comparisons (small/medium and medium/large). Based on Caporali et al. 1994, development of the male flowers' pistil and carpels (gynoecium) ceased when the buds reached 1.3 mm in length. Some of the genes expressed by the gynoecium of the flower would still be expressed or the transcripts remained in the medium buds but not the large buds (Caporali et al., 1994). Therefore, both male and female organ development programs are expressed in small buds (< 1 mm), the female organ development program is shut down in most medium (1-2 mm) and all large (> 2 mm) buds. Having more replicates and from different lines including a YY supermale could reveal more genes that are differentially expressed.

The expression level of *SOFF* is too low for 1X coverage given 30 million RNA seq reads derived from full flower buds. RT-PCR would likely not be able to detect the splice variant of *SOFF* due to its' low expression and would be hard to distinguish from *SOFF-Like* due to sequence similarity. Deep sequencing using single cell RNA-seq might be able to detect the presence of the *SOFF* and its hypothesized alternative transcript assuming they are only expressed in a few cells. However, *SOFF* expression was not detected in *in situ* experiments (Harkess et al., 2020) suggesting it is expressed at very low levels in a narrow set of cells and developmental stages.

StringTie assemblies of the RNA seq reads indicate that the current *A. officinalis* genome annotation is missing a significant number of gene models. This finding is consistent with ChIP seq analysis of histone modification associated with transcription start sites (Mendieta et al., 2021). Refinement of an *A. officinalis* genome assembly and annotation is underway (Harkess and Leebens-Mack labs, in progress).

CHAPTER 2

TRANSFORMING *ASPARAGUS OFFICINALIS* L. WITH *AGROBACTERIUM TUMEFACIENS*

Introduction

Most genomic and biotechnology work on monocots has been done in the grass family, *Poaceae*. However, *Poaceae* genome characteristics are not fully representative of many other monocots, including *Asparagus* (Kuhl et al., 2005; Vitte et al., 2013). While rice, wheat, corn, and other crops in the grass family are understandably where most genetic engineering is being done (Liu et al., 2021), garden asparagus (*Asparagus officinalis*) was the first monocot plant to be transformed by *Agrobacterium* (Bytebier et al., 1987; Hernalsteens et al., 1984). Pioneering work done on asparagus in the 1980s and 1990s inspired development of transformation protocols in *Poaceae*, but little has been done with asparagus transformation since (Anido & Cointry, 2008).

To better understand the seven genes in the nonrecombining region of the Y chromosome effects on sex determination, CRISPR-Cas9 can be utilized to edit each gene individually or multiple genes at one time. A robust transformation protocol is needed to optimize recovery of transformed plants with available asparagus genotypes. The time it takes to go from cultured asparagus cells (derived from cuttings or callus) to flowering spears is >6 months. In order to assess the effect of edited genes in the nonrecombining region of the Y chromosome have on sex determination, flowers must be observed to see whether the androecium (male reproductive structures) and gynoecium (female reproductive structures) properly develop. Therefore, before proceeding with CRISPR gene editing experiments to investigate gene function with respect to sex determination, we want to optimize transformation and shoot and root induction efficiencies for our available genotypes.

When developing transformation protocols for under-studied species, having target plants express reporters that are easily visualized helps to quickly assess transformation efficiency. Some commonly used indicators of plant transformation are β-glucuronidase (GUS), green fluorescent protein (GFP), RUBY or knockouts of the native *phytoene desaturase (PDS)* gene resulting in achlorophyllous, white plants (Chiu et al., 1996; He et al., 2020; Helliwell et al., 2002; Jefferson et al., 1987; Naing et al., 2019). Each indicator has its advantages and disadvantages, however, GFP and RUBY do not destroy the tissue of the plant.

GUS is stable and safe to humans and the environment. It does not affect plant growth or development when it has been transformed. The GUS assay destroys the tissue when the reagents are added to detect for its' presence. However, the reagents are cheaper in cost than with other reporter's reagents or needed equipment. A concern with GUS is that is hard to completely eliminate false positives (Xiong et al., 2011).

GFP allows for viewing of live tissues over time, requires expensive microscopes to visualize if it is being expressed, and scoring can be hindered by tissue auto fluorescence (Xiong et al., 2011). With *PDS* knockout experiments, it can be hard to tell whether achlorophyllous growth in callus and shoots is a consequence of stress or loss of PDS function. Expressing anthocyanins in plants is not easy since it requires multiple enzymes and can vary between plants. Vectors containing RUBY encoding betalain biosynthetic pathway genes, turn parts or the entire plant red in color. There are only three enzymatic reactions in the betalain pathway. Some plants naturally express betalain including dragonfruit, beets, and swiss chard. RUBY cannot be used for plants that have tissue that is already red or dark in color (He et al., 2020).

Previous asparagus transformation protocols used a myriad of selection antibiotics and two different strains of *Agrobacterium* (Table 1). Most of the transformation rates reported were based on the number of clones produced per gram of embryogenic tissue (Bytebier et al., 1987; Cabrera-Ponce et al., 1997; Chen et al., 2019; Hernalsteens et al., 1984). Cabrera-Ponce et al. 1997 used a gene gun instead of agrobacterium. Building upon these protocols, I aimed to develop optimized transformation, propagation, and shoot induction protocols in garden asparagus.

Table 1. Examples of previous garden asparagus transformation experiments done from literature.

	Agro	Tissue used	Selection (mg/L)	Highest transformation rate
Chen et al. 2019	EHA105	embryo	PPT<20	21%
Bytebier et al. 1987	C58	spears	Kan 25	
Hernalsteens et al. 1984	C58	stem fragments		
Cabrera-Ponce et al. 1997	NA	embryogenic callus	Hyg 25 , PPT 4	

Methods

Callus Generation

Garden asparagus seed was sterilized with a 0.3% bleach solution for 10 minutes and germinated on MS media in 16oz clear plastic cups with dome lids. After germination, plants were moved to ½ MS media and subcultured every few weeks. When spears or roots became overcrowded within each culture, they were trimmed back manually. Spears were cut into 5-10 mm pieces and cladodes were removed. Two or three evenly spaced cuts were made into these subsections, about halfway through to generate callus tissue. The spear pieces were then placed on a plate with CIM (Callus Induction Media) (Figure 6).

Figure 6. Garden asparagus in tissue culture. On the left is the cup that the germinated seed is grown in, about 3 months old. On the right is the plate where the spear pieces have been added to CIM.

Transformation

Initial transformations used callus tissue from two different genotypes as the starting material (75 calli of each). *Agrobacterium* (strain C58/pMP90) with the plasmids containing PDS or YFP (Yellow Fluorescent Protein) inserted were provided by the Tsai lab and used for initial transformations. This set of transformations resulted in low transformation efficiency. Callus is composed of dense clusters of cells which can be difficult for agrobacterium to penetrate. Chimeric transformations are most likely to occur when transforming callus tissue. The second set of transformations were started using spear pieces rather than established callus tissue. Other systems (*Populus*) have been successful using leaf tissue for transformation. Transformation using spear pieces takes longer because it takes about two months to develop into callus.

The third iteration of transformations used mEGFP and RUBY for spear tips which was then repeated with callus as the starting material for comparison.

The final transformations used free use free-use GFP (fuGFP) which has a brighter signal level than mEGFP and callus was used as starting material due to time constraints (Coleman and Somerville, in prep) (Table 2).

Media recipes were adapted from Reddy et al., 2007 (Appendix A, Table 10). The following protocol is a modified version of the 717-transformation protocol used in the Tsai lab (http://aspendb.uga.edu/downloads/717-transformation.pdf). Spear pieces were arranged with the cut side up on the CIM plate in 4 rows of 5. A P1000 pipette was set to 1mL, and a drop of agrobacterium was applied to each spear piece. After sitting for 10 minutes, the petri dish was angled, and any remaining agrobacterium liquid left was removed with the P1000. Plates were then wrapped in parafilm and placed in a box, with a closed lid, in the growth chamber. A control plate with 20 spear pieces that did not have agrobacterium applied was wrapped in parafilm and placed in the same box.

When starting transformations with callus, callus is cut into small pieces that are at least 3 mm on each side and no more than 9 mm on a side. After all the callus pieces were cut, they were placed in a flask with the subcultured agrobacteria, making sure that all the callus was covered. The flasks were put on a shaker (~250 rpm) at 28^{o}C for 10 minutes. The flasks were taken back into the sterile hood and the callus was removed by pouring into a strainer over a beaker. Callus pieces were then placed on a CIM plate and wrapped in parafilm and placed in a box, with a closed lid, in the growth chamber. Transformations from February 23, 2022, had 60% less Acetosyringone and were placed in a 19^{o}C incubator for co-cultivation instead of in the growth chamber to try to improve transformation rate. After co-cultivation for two days on CIM, callus was removed and washed five times, the same as with the spear pieces, before being placed on CIM media with selective antibiotics.

Half of the spear pieces or callus on the control plate were moved to a CIM plate with Hygromycin and Timentin to serve as a negative control. Plates were then wrapped in parafilm and placed in a box, with a closed lid, in the growth chamber. Every week, images were taken of the plates to record growth.

About every two weeks spear pieces and callus were moved to a fresh CIM plate with Hygromycin and Timentin (Tables 3 and 4). The control plate was moved to a fresh CIM plate on the same day. Once callus developed on the plates, they were moved to SIM (Shoot Induction Media) with Hygromycin and Timentin. After one week on SIM, the plates were moved under grow lights.

Callus transformations from February 23, 2022, were cut into smaller pieces five days after being washed and placed on a plate with CIM TH/ (Table 3).

Table 2. Transformation experiments done including plasmid used, tissue used, and amount of tissue used.

Date of transformation	Plasmid used	Genotype	Tissue used	Tissue amount
9/29/2021	RUBY	WB	spear pieces	53
9/29/2021	RUBY	G	spear pieces	60
10/15/2021	RUBY	WB	spear pieces	58
10/15/2021	RUBY	G	spear pieces	60
11/10/2021	mEGFP	WB	spear pieces	60
11/10/2021	mEGFP	G	spear pieces	60
12/12/2021	RUBY	WB	spear pieces	64
12/12/2021	RUBY	G	spear pieces	60
12/12/2021	RUBY	WB	callus	64
12/12/2021	RUBY	G	callus	64
2/23/2022	fuGFP	WB	callus	52
2/23/2022	fuGFP	G	callus	50
2/23/2022	RUBY	WB	callus	50
2/23/2022	RUBY	G	callus	50

Table 3. Differing amounts of Hygromycin (H or Hyg) used as selective antibiotic during transformations. Amount of Timentin (T) used in plates.

	T	Hyg
TH	200 mg/L	10 mg/L
TH+	200 mg/L	100 mg/L
TH/	200 mg/L	25 mg/L

Table 4. How long tissues were on selective antibiotics throughout transformation experiments.

Date of transformation	Selection	Time	Selection	Time	Selection	Time	Selection	Time
9/29/2021	Spec	7 days	Hyg	61 days	Hyg +	35 days	Hyg/	44 days
9/29/2021	Spec	7 days	Hyg	61 days	Hyg +	35 days	Hyg/	44 days
10/15/2021	Hyg	52 days	Hyg +	35 days	Hyg/	44 days		
10/15/2021	Hyg	52 days	Hyg +	35 days	Hyg/	44 days		
11/10/2021	Hyg	27 days	Hyg +	35 days	Hyg/	75 days		
11/10/2021	Hyg	27 days	Hyg +	35 days	Hyg/	75 days		
12/12/2021	Hyg +	29 days	Hyg/	75 days				
12/12/2021	Hyg +	29 days	Hyg/	75 days				
12/12/2021	Hyg +	29 days	Hyg/	75 days				
12/12/2021	Hyg +	29 days	Hyg/	75 days				
2/23/2022	Hyg/	60 days						
2/23/2022	Hyg/	60 days						
2/23/2022	Hyg/	60 days						
2/23/2022	Hyg/	60 days						

Results/ Discussion

The *PDS* CRISPR knockout treatments did result in white plants; however, amplicon sequencing confirmed that these plants did not contain any edits. The white plants are likely a result of the stress caused by tissue culture rather than a successful transformation event. One YFP transformant had YFP expressed in the nucleus (Figure 7), but the callus did not grow any shoots nor roots. The YFP should have been expressed in the plasma membrane.

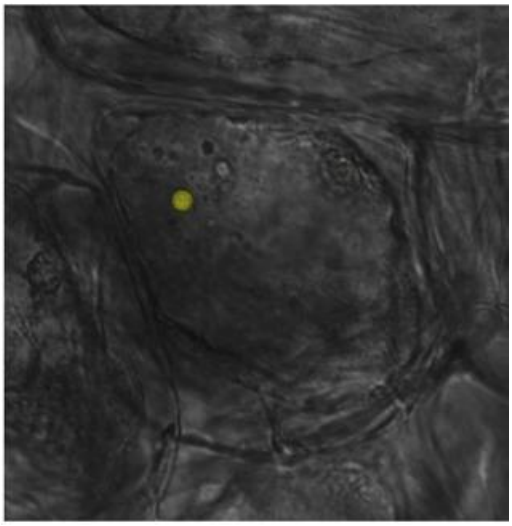


Figure 7. YFP expressed in the nucleus of a garden asparagus callus cell. Image taken with confocal microscope.

Only one callus from the December 12, 2021 transformation showed clear expression of RUBY in the WB genotype (Figure 8, Table 2). Another set of experiments were done on February 23, 2022 to see if the results could be replicated. The concentration of the Hygromycin, the transformation selective antibiotic, to be used was not known in the initial experiments which is why there was a change in the concentrations (Tables 3 and 4). The untransformed tissue was growing on the CIM plate with the lowest concentration of Hygromycin and was increased to prevent the chance of a false positive appearing on the transformed plates. When the higher concentration of Hygromycin was found to be killing the control tissue in a couple days, the concentration was lowered. The last set of experiments have been kept on this intermediate amount of Hygromycin concentration. The concentration of Timentin was kept the same throughout. The Hygromycin resistance was provided by the plasmid that the garden asparagus tissue was transformed with. Only the one transformed piece of callus that expressed RUBY was moved to SIM. At this time, it has not developed any red shoots but has started to sprout some green shoots.

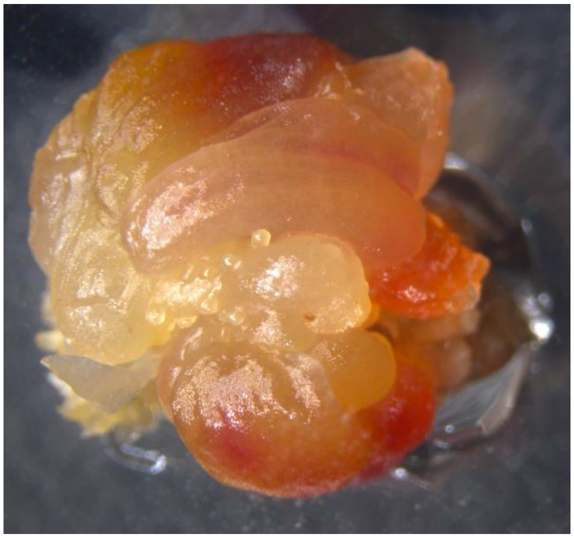


Figure 8. Garden asparagus callus expressing the RUBY construct.

Table 5. Summary of results of transformation experiments. # of tissue that grew does not include tissue that developed into shoots but any tissue that increased in size after being placed on CIM plates with antibiotics.

Date of transformation	Plasmid used	Genotype	Tissue Used	# of tissue used	# of tissue that grew	Notes
9/29/2021	RUBY	WB	spear pieces	53	11	
9/29/2021	RUBY	G	spear pieces	60	5	
10/15/2021	RUBY	WB	spear pieces	58	11	
10/15/2021	RUBY	G	spear pieces	60	1	Agro contamination only 4 made it past 3 weeks
11/10/2021	GFP	WB	spear pieces	60	9	
11/10/2021	GFP	G	spear pieces	60	2	
12/12/2021	RUBY	WB	spear pieces	64	4	
12/12/2021	RUBY	G	spear pieces	60	5	
12/12/2021	RUBY	WB	callus	64	20	
12/12/2021	RUBY	G	callus	64	13	
2/23/2022	fuGFP	WB	callus	105	10	
2/23/2022	fuGFP	G	callus	110	3	
2/23/2022	RUBY	WB	callus	115	15	
2/23/2022	RUBY	G	callus	107	8	

The results of my transformations have a low rate of transformation, only one of 64 agrobacterium-treated callus, 1.6%, exhibited a clear transformation phenotype (Figure 8). Some of the callus from the February 23, 2022 transformation may express fuGFP though the levels currently are just a little higher than autofluorescence. Other transformation experiments done with garden asparagus have had better rates but with different genotypes as well as different strains of agrobacterium (Table 1). More transformation work would need to be done with the lines used in my experiments before CRISPR-Cas9 is used to edit genes in the nonrecombining region of the Y chromosome.

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APPENDIX A

Supplemental Information

Table 6. Reads and basepairs from RNA-seq. NJ1, NJ2, and NJ3 are the male plants. F is the female plant.

Plant	Bud size	Reads	Basepairs
NJ1	<1 mm	40,396,876	6,099,928,276
NJ1	1-2 mm	37,021,820	5,590,294,820
NJ1	3-5 mm	6,264,358	945,918,058
NJ2	<1 mm	42,170,270	6,367,710,770
NJ2	1-2 mm	41,171,568	6,216,906,768
NJ2	3-5 mm	32,054,860	4,840,283,860
NJ3	<1 mm	42,388,520	6,400,666,520
NJ3	1-2 mm	35,083,718	5,297,641,418
NJ3	3-5 mm	35,764,894	5,400,498,994
F	<1 mm	36,848,488	5,564,121,688
F	1-2 mm	6,776,376	1,023,232,776
F	2-3 mm	39,904,596	6,025,593,996

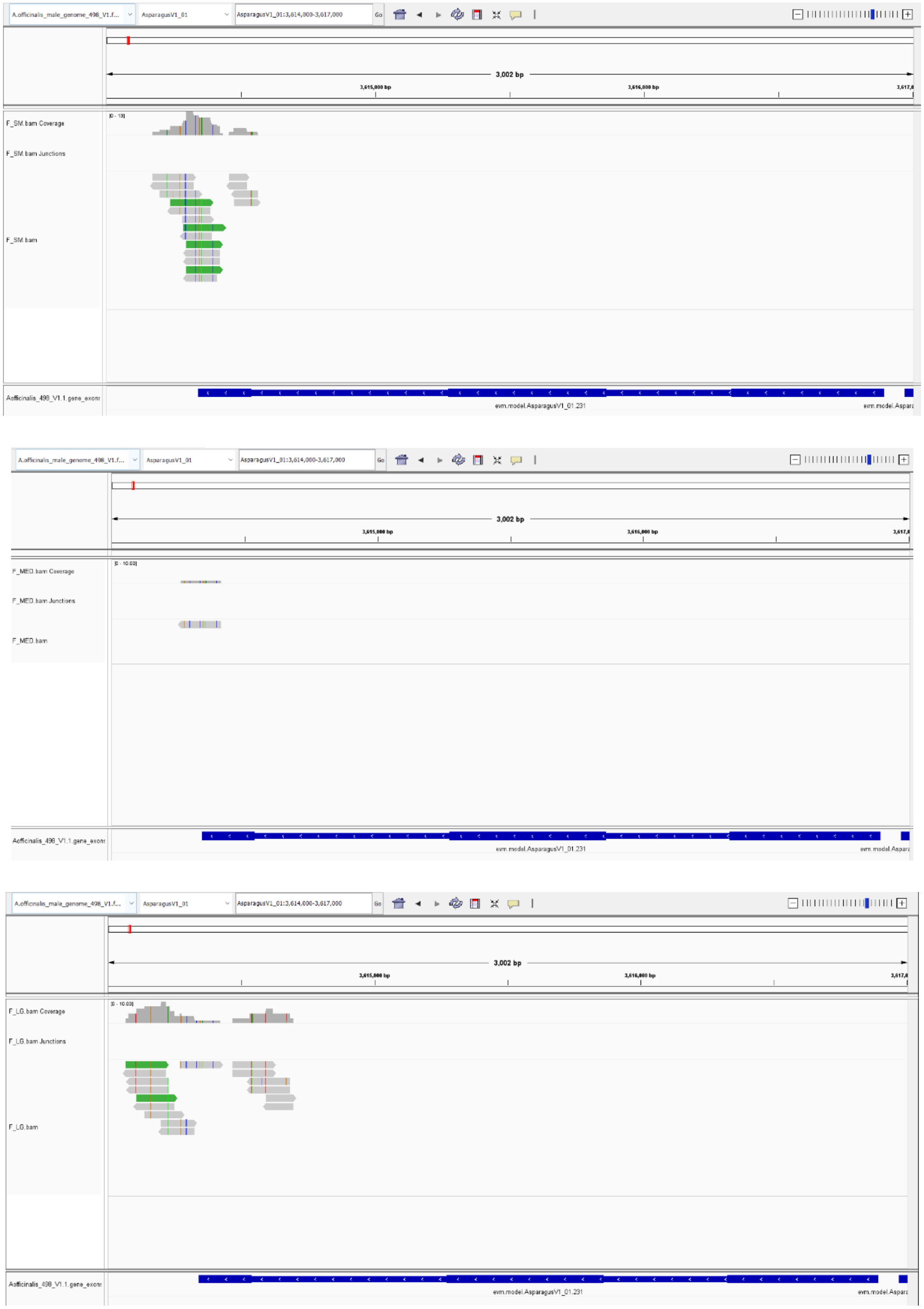

Figure 9. IGV view of female RNA-seq reads. Top is small buds, middle is medium buds and bottom is large buds. SOFF gene model in blue at bottom of each image.

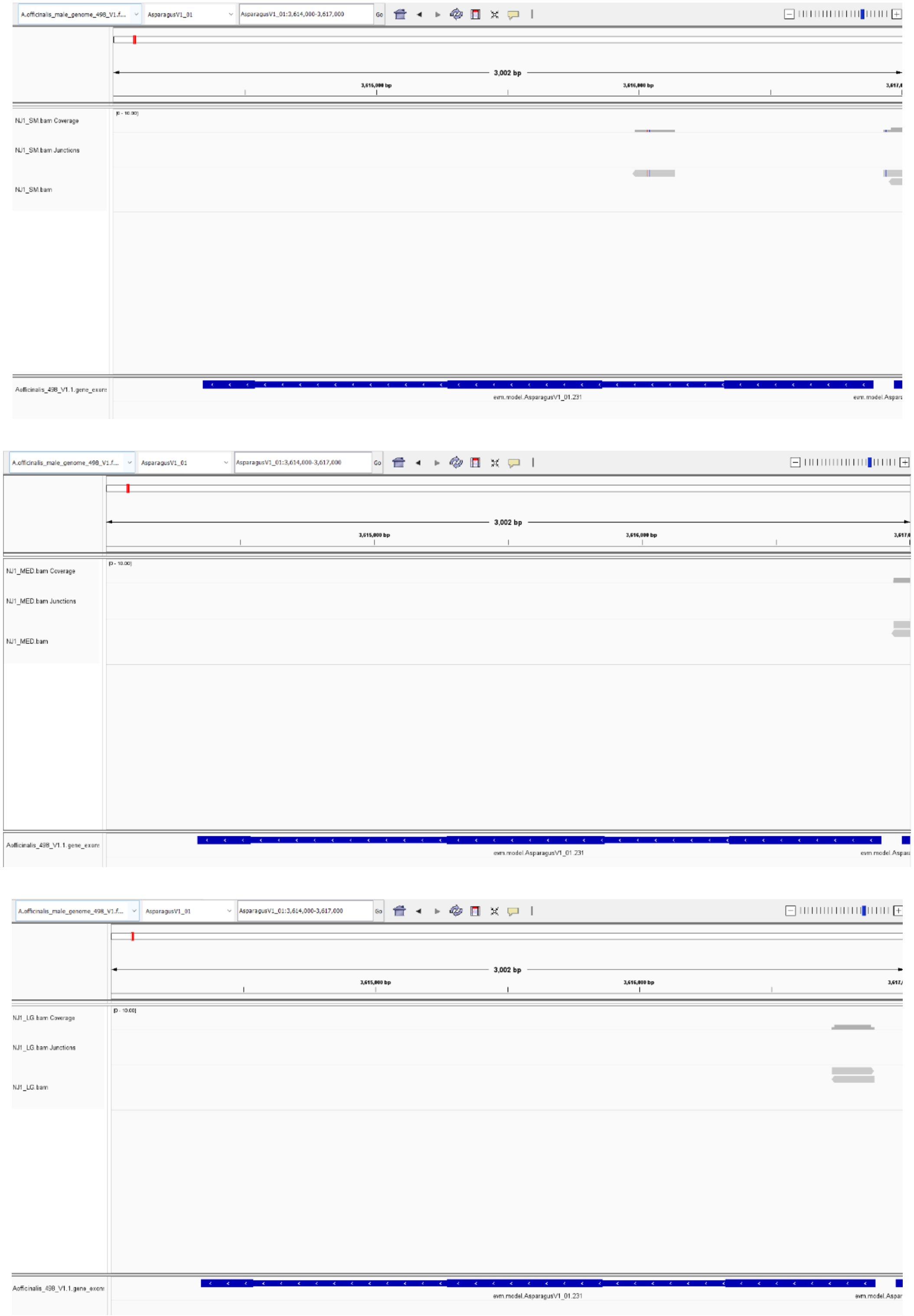

Figure 10. IGV view of Male1 RNA-seq reads. Top is small buds, middle is medium buds and bottom is large buds. SOFF gene model in blue at bottom of each image.

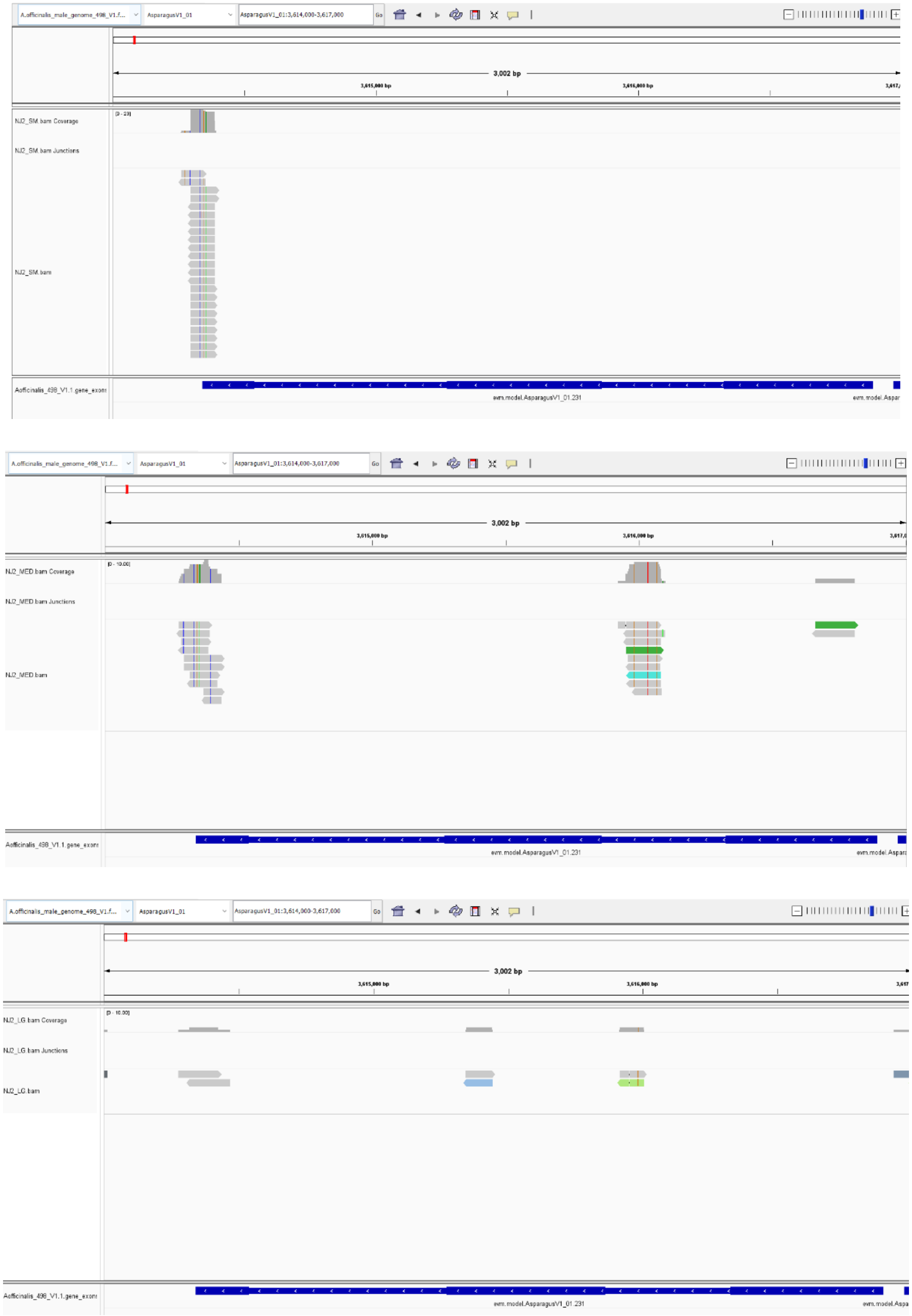

Figure 11. IGV view of Male2 RNA-seq reads. Top is small buds, middle is medium buds and bottom is large buds. SOFF gene model in blue at bottom of each image.

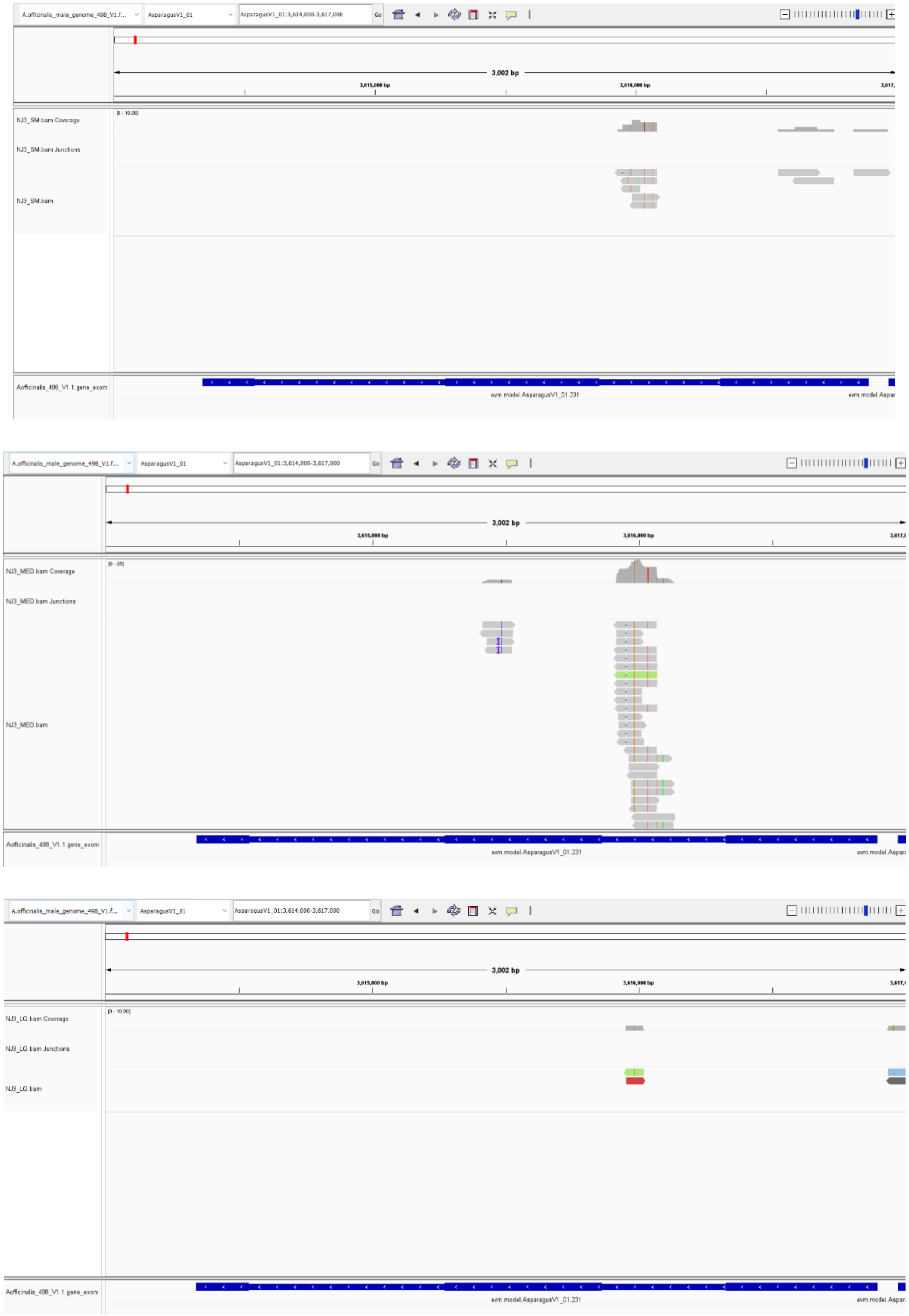

Figure 12. IGV view of Male3 RNA-seq reads. Top is small buds, middle is medium buds and bottom is large buds. SOFF gene model in blue at bottom of each image.

Table 7. List of differentially expressed genes with gene models in the current annotation. Significantly differentially expressed genes identified with a p-value < 0.5 and a false discovery rate (q-value) < 0.5 were only seen in the comparison of small and large male flower buds.

Gene name	Gene function	P value	Q value	Fold Change
evm.model.AsparagusV1_08.3103	(1 of 3) PTHR10168//PTHR10168:SF57 - GLUTAREDOXIN // GLUTAREDOXIN-C13-RELATED	4.26E-06	0.02233	0.041496
evm.model.AsparagusV1_01.3237	(1 of 1) PF07714//PF13947 - Protein tyrosine kinase (Pkinase_Tyr) // Wall-associated receptor kinase galacturonan-binding (GUB_WAK_bind)	3.38E-05	0.039005	6.000127
evm.model.AsparagusV1_10.1589	(1 of 4) 2.8.2.15 - Steroid sulfotransferase	4.58E-05	0.039005	0.172684
evm.model.AsparagusV1_02.45	(1 of 1) K14682 - amino-acid N-acetyltransferase (argAB)	5.97E-05	0.039005	7.966793
evm.model.AsparagusV1_02.187	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	6.33E-05	0.039005	2.580593
evm.model.AsparagusV1_08.2435	Missing annotation	7.32E-05	0.039005	3.364404
evm.model.AsparagusV1_03.2339	(1 of 12) K09285 - AP2-like factor, ANT lineage (OVM, ANT)	7.46E-05	0.039005	58.16624
evm.model.AsparagusV1_04.1413	Missing annotation	8.20E-05	0.039005	15.09485
evm.model.AsparagusV1_05.96	(1 of 1) K09494 - T-complex protein 1 subunit beta (CCT2)	9.14E-05	0.039005	2.960945
evm.model.AsparagusV1_02.861	(1 of 5) PTHR24298:SF47 - CYTOCHROME P450 77A4-RELATED	9.68E-05	0.039005	0.008098
evm.model.AsparagusV1_07.1409	(1 of 3) PTHR10992:SF811 - ALPHA/BETA-HYDROLASES SUPERFAMILY PROTEIN	0.000107	0.039005	0.042334
evm.model.AsparagusV1_03.2371	(1 of 2) K13195 - cold-inducible RNA-binding protein (CIRBP)	0.000111	0.039005	7.742982

evm.model.AsparagusV1_03.2816	(1 of 1) PTHR11390//PTHR11390:SF21 - PROKARYOTIC DNA TOPOISOMERASE // DNA TOPOISOMERASE 3-ALPHA	0.000121	0.039005	5.393444
evm.model.AsparagusV1_08.1007	Missing annotation	0.000122	0.039005	5.749416
evm.model.AsparagusV1_04.2235	(1 of 26) K04733 - interleukin-1 receptor-associated kinase 4 (IRAK4)	0.000124	0.039005	0.168054
evm.model.AsparagusV1_07.3202	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	0.000128	0.039005	2.61653
evm.model.AsparagusV1_05.2823	(1 of 1) PTHR12547//PTHR12547:SF63 - CCCH ZINC FINGER/TIS11-RELATED // ZINC FINGER CCCH DOMAIN-CONTAINING PROTEIN 37-RELATED	0.000136	0.039005	6.196761
evm.model.AsparagusV1_08.3375	(1 of 1) PTHR31934:SF2 - RNA-DIRECTED DNA METHYLATION 4	0.000138	0.039005	5.564177
evm.model.AsparagusV1_05.285	Missing annotation	0.000145	0.039005	0.031139
evm.model.AsparagusV1_03.2414	(1 of 2) K12831 - splicing factor 3B subunit 4 (SF3B4, SAP49)	0.00015	0.039005	3.653173
evm.model.AsparagusV1_07.2206	(1 of 19) PF00560//PF13855 - Leucine Rich Repeat (LRR_1) // Leucine rich repeat (LRR_8)	0.000152	0.039005	2.342846
evm.model.AsparagusV1_03.2370	Missing annotation	0.000152	0.039005	2.373308
evm.model.AsparagusV1_05.3088	(1 of 20) PF05553 - Cotton fibre expressed protein (DUF761)	0.000167	0.039551	17.56108
evm.model.AsparagusV1_03.655	Missing annotation	0.000172	0.039551	14.55061
evm.model.AsparagusV1_07.60	(1 of 2) KOG3591 - Alpha crystallins	0.000177	0.039551	90.67011
evm.model.AsparagusV1_09.179	(1 of 2) PF02496 - ABA/WDS induced protein (ABA_WDS)	0.000181	0.039551	0.077506
evm.model.AsparagusV1_06.542	(1 of 1) PTHR31495:SF1 - PEROXYGENASE 4-RELATED	0.000186	0.039551	0.048406
evm.model.AsparagusV1_07.335	(1 of 1) PTHR24326:SF115 - WUSCHEL-RELATED HOMEOBOX 3	0.000188	0.039551	6.771245

evm.model.AsparagusV1_Unassigned.1007	(1 of 3) PTHR24012:SF376 - POLYPYRIMIDINE TRACT-BINDING PROTEIN 3	0.000195	0.039551	2.723632
evm.model.AsparagusV1_04.832	(1 of 2) K13249 - translocon-associated protein subunit alpha (SSR1)	0.000196	0.039551	2.191643
evm.model.AsparagusV1_04.2769	Missing annotation	0.000204	0.039551	4.622212
evm.model.AsparagusV1_07.842	(1 of 2) K14317 - nuclear pore complex protein Nup214 (NUP214, CAN)	0.000217	0.040163	0.11134
evm.model.AsparagusV1_08.1445	(1 of 471) PF13041 - PPR repeat family (PPR_2)	0.000227	0.040432	2.579763
evm.model.AsparagusV1_04.191	(1 of 1) PTHR32246//PTHR32246:SF16 - FAMILY NOT NAMED // CALCIUM-DEPENDENT LIPID-BINDING DOMAIN-CONTAINING PROTEIN-RELATED	0.000237	0.040432	0.007396
evm.model.AsparagusV1_05.3063	(1 of 1) PTHR33210:SF6 - PROTODERMAL FACTOR 1	0.000266	0.041607	155.0996
evm.model.AsparagusV1_03.1260	Missing annotation	0.000267	0.041607	433.7503
evm.model.AsparagusV1_04.600	Missing annotation	0.000272	0.041607	0.008295
evm.model.AsparagusV1_07.1517	(1 of 1) K17545 - serine/threonine-protein kinase ULK4 [EC:2.7.11.1] (ULK4)	0.000275	0.041607	9.764564
evm.model.AsparagusV1_01.2910	(1 of 1) PTHR33052//PTHR33052:SF22 - FAMILY NOT NAMED // F15H18.19	0.000326	0.041992	0.63278
evm.model.AsparagusV1_05.281	(1 of 1) K09651 - rhomboid domain-containing protein 1 [EC:3.4.21.-] (RHBDD1)	0.000338	0.041992	1.874404
evm.model.AsparagusV1_08.2761	(1 of 5) PTHR23500:SF47 - SUGAR TRANSPORT PROTEIN 10-RELATED	0.000338	0.041992	2.084212
evm.model.AsparagusV1_04.674	(1 of 1) PF00069//PF13837 - Protein kinase domain (Pkinase) // Myb/SANT-like DNA-binding domain (Myb_DNA-bind_4)	0.000341	0.041992	5.281141
evm.model.AsparagusV1_03.2770	(1 of 1) K16253 - DNA-directed RNA polymerase IV and V subunit 7 (NRPD7, NRPE7)	0.000352	0.041992	3.329917

evm.model.AsparagusV1_06.34	(1 of 4) PTHR31744:SF5 - NAC DOMAIN-CONTAINING PROTEIN 21/22-RELATED	0.000361	0.041992	0.151737
evm.model.AsparagusV1_05.137	(1 of 72) 3.1.1.11 - Pectinesterase / Pectin methylesterase	0.00037	0.041992	0.000817
evm.model.AsparagusV1_02.2086	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	0.000379	0.041992	3.525688
evm.model.AsparagusV1_02.655	(1 of 1) PTHR11785//PTHR11785:SF370 - AMINO ACID TRANSPORTER // SUBFAMILY NOT NAMED	0.000388	0.041992	13.66475
evm.model.AsparagusV1_05.2481	(1 of 8) 3.6.3.27 - Phosphate-transporting ATPase / ABC phosphate transporter	0.00039	0.041992	0.540288
evm.model.AsparagusV1_04.1092	Missing annotation	0.000396	0.041992	0.000673
evm.model.AsparagusV1_01.3093	(1 of 3) K07393 - putative glutathione S-transferase (ECM4)	0.000398	0.041992	15.91989
evm.model.AsparagusV1_08.949	(1 of 37) PF02298 - Plastocyanin-like domain (Cu_bind_like)	0.000401	0.041992	3.97565
evm.model.AsparagusV1_08.717	(1 of 8) PF01918 - Alba (Alba)	0.000429	0.043165	6.367809
evm.model.AsparagusV1_02.862	Missing annotation	0.000449	0.043821	0.039542
evm.model.AsparagusV1_07.2823	Missing annotation	0.000465	0.043821	1.136406
evm.model.AsparagusV1_08.2824	(1 of 1) K09500 - T-complex protein 1 subunit theta (CCT8)	0.000468	0.043821	3.674641
evm.model.AsparagusV1_02.1930	(1 of 1) PTHR31407:SF14 - PSBP DOMAIN-CONTAINING PROTEIN 6, CHLOROPLASTIC	0.000473	0.043821	11.05527
evm.model.AsparagusV1_02.157	(1 of 11) PF00314 - Thaumatin family (Thaumatin)	0.000477	0.043821	19.55096
evm.model.AsparagusV1_03.3109	(1 of 3) PF07647 - SAM domain (Sterile alpha motif) (SAM_2)	0.00048	0.043821	0.260931
evm.model.AsparagusV1_08.3197	Missing annotation	0.000481	0.043821	0.001657
evm.model.AsparagusV1_05.3246	(1 of 1) K03011 - DNA-directed RNA polymerase II subunit RPB3 (RPB3, POLR2C)	0.000493	0.044453	3.178052

evm.model.AsparagusV1_08.1049	(1 of 1) K09497 - T-complex protein 1 subunit epsilon (CCT5)	0.000504	0.044453	5.687675
evm.model.AsparagusV1_04.3123	(1 of 3) PTHR31223//PTHR31223:SF17 - FAMILY NOT NAMED // CYTOKININ RIBOSIDE 5'-MONOPHOSPHATE PHOSPHORIBOHYDROLASE LOG3-RELATED	0.000511	0.044453	2.861444
evm.model.AsparagusV1_07.3951	(1 of 9) PTHR12341 - 5'->3' EXORIBONUCLEASE	0.000526	0.044705	5.862379
evm.model.AsparagusV1_03.281	(1 of 3) PTHR31279//PTHR31279:SF3 - FAMILY NOT NAMED // (PHI-1) PROTEIN, PUTATIVE-RELATED	0.000536	0.044705	0.626471
evm.model.AsparagusV1_04.363	(1 of 9) PF05340 - Protein of unknown function (DUF740) (DUF740)	0.000538	0.044705	0.13505
evm.model.AsparagusV1_Unassigned.45	(1 of 9) PF07714//PF11721 - Protein tyrosine kinase (Pkinase_Tyr) // Di-glucose binding within endoplasmic reticulum (Malectin)	0.000564	0.044974	2.783966
evm.model.AsparagusV1_08.938	(1 of 1) PTHR27002//PTHR27002:SF42 - FAMILY NOT NAMED // G-TYPE LECTIN S-RECEPTOR-LIKE SERINE/THREONINE-PROTEIN KINASE SD3-1	0.000578	0.044974	3.980905
evm.model.AsparagusV1_03.1847	(1 of 2) PTHR21337//PTHR21337:SF7 - PHOSPHO-2-DEHYDRO-3-DEOXYHEPTONATE ALDOLASE 1, 2 // SUBFAMILY NOT NAMED	0.00058	0.044974	1.372577
evm.model.AsparagusV1_07.1801	(1 of 1) K09496 - T-complex protein 1 subunit delta (CCT4)	0.000585	0.044974	4.616192
evm.model.AsparagusV1_08.689	(1 of 3) PTHR23012//PTHR23012:SF54 - MEMBRANE ASSOCIATED RING FINGER // SUBFAMILY NOT NAMED	0.000587	0.044974	4.392005

evm.model.AsparagusV1_03.394	(1 of 5) PF04759 - Protein of unknown function, DUF617 (DUF617)	0.00059	0.044974	0.043613
evm.model.AsparagusV1_09.301	(1 of 3) PTHR35753//PTHR35753:SF1 - FAMILY NOT NAMED // PROLINE-RICH FAMILY PROTEIN	0.0006	0.045119	2.285997
evm.model.AsparagusV1_08.401	(1 of 2) PTHR24128:SF13 - E3 UBIQUITIN-PROTEIN LIGASE XBAT33	0.000612	0.045119	0.141671
evm.model.AsparagusV1_02.1522	(1 of 1) PTHR12565:SF152 - TRANSCRIPTION FACTOR BHLH63-RELATED	0.000618	0.045119	0.294402
evm.model.AsparagusV1_04.1308	(1 of 2) PTHR12613 - ERO1-RELATED	0.000624	0.045119	2.122358
evm.model.AsparagusV1_03.843	Missing annotation	0.000632	0.045223	0.065052
evm.model.AsparagusV1_08.2249	(1 of 44) PF01535//PF13041//PF13812 - PPR repeat (PPR) // PPR repeat family (PPR_2) // Pentatricopeptide repeat domain (PPR_3)	0.000636	0.045321	0.370838
evm.model.AsparagusV1_04.2442	Missing annotation	0.000652	0.045988	16.25222
evm.model.AsparagusV1_02.1347	(1 of 1) K12876 - RNA-binding protein 8A (RBM8A, Y14)	0.000666	0.045988	2.365802
evm.model.AsparagusV1_06.1928	(1 of 5) K10752 - histone-binding protein RBBP4 (RBBP4, HAT2, CAF1, MIS16)	0.000666	0.045988	254.9799
evm.model.AsparagusV1_08.390	Missing annotation	0.000667	0.045988	1294.717
evm.model.AsparagusV1_03.1126	Missing annotation	0.000678	0.046224	76.64257
evm.model.AsparagusV1_03.2920	(1 of 2) K08099 - chlorophyllase (E3.1.1.14)	0.000682	0.046224	13.02288
evm.model.AsparagusV1_05.145	(1 of 6) PF06273 - Plant specific eukaryotic initiation factor 4B (eIF-4B)	0.000682	0.046224	11.21736
evm.model.AsparagusV1_03.27	(1 of 4) 3.1.27.1 - Ribonuclease T(2) / Ribonuclease T2	0.0007	0.046461	0.015186
evm.model.AsparagusV1_07.1014	(1 of 2) PTHR12506:SF27 - ZINC FINGER CCCH DOMAIN-CONTAINING PROTEIN 34-RELATED	0.000703	0.046461	3.36604
evm.model.AsparagusV1_01.2053	(1 of 1) 3.1.3.75//3.6.1.1 - Phosphoethanolamine/phosphocholine	0.000719	0.046477	0.294223

	phosphatase / PHOSPHO1 // Inorganic diphosphatase / Pyrophosphate phosphohydrolase			
evm.model.AsparagusV1_05.2548	(1 of 2) PF08553 - VID27 cytoplasmic protein (VID27)	0.000727	0.0466	31.70693
evm.model.AsparagusV1_09.44	(1 of 66) PF01535//PF13041//PF14432 - PPR repeat (PPR) // PPR repeat family (PPR_2) // DYW family of nucleic acid deaminases (DYW_deaminase)	0.000771	0.047507	1.753694
evm.model.AsparagusV1_04.2010	(1 of 1) K16278 - E3 ubiquitin-protein ligase HOS1 [EC:6.3.2.19] (HOS1)	0.000785	0.047507	3.52258
evm.model.AsparagusV1_08.3265	(1 of 4) K02885 - large subunit ribosomal protein L19e (RP-L19e, RPL19)	0.000854	0.047507	3.333912
evm.model.AsparagusV1_03.2854	(1 of 2) PTHR31250:SF4 - CALMODULIN-BINDING FAMILY PROTEIN-RELATED	0.000864	0.047507	0.00318
evm.model.AsparagusV1_05.249	Missing annotation	0.000867	0.047507	0.335226
evm.model.AsparagusV1_Unassigned.1002	(1 of 29) PF01535//PF12854//PF13041 - PPR repeat (PPR) // PPR repeat (PPR_1) // PPR repeat family (PPR_2)	0.000899	0.047507	4.115509
evm.model.AsparagusV1_04.2768	(1 of 3) PTHR31614//PTHR31614:SF5 - FAMILY NOT NAMED // ALLERGEN-LIKE PROTEIN BRSN20-RELATED	0.000908	0.047507	0.001933
evm.model.AsparagusV1_03.3207	(1 of 4) K09377 - cysteine and glycine-rich protein (CSRP)	0.000911	0.047507	0.232721
evm.model.AsparagusV1_03.2128	Missing annotation	0.000924	0.047507	0.020192
evm.model.AsparagusV1_01.3210	Missing annotation	0.000926	0.047507	0.214525
evm.model.AsparagusV1_07.962	Missing annotation	0.000931	0.047507	1.423388
evm.model.AsparagusV1_05.4	(1 of 1) PTHR33021:SF41 - EARLY NODULIN-LIKE PROTEIN 18	0.00096	0.047507	0.005998
evm.model.AsparagusV1_02.1570	(1 of 1) 3.4.23.25 - Saccharopepsin / Yeast endopeptidase A	0.000967	0.047507	0.006805

evm.model.AsparagusV1_04.2891	(1 of 3) PTHR22950//PTHR22950:SF295 - AMINO ACID TRANSPORTER // AMINO ACID PERMEASE 2	0.000969	0.047507	0.167544
evm.model.AsparagusV1_08.871	(1 of 3) PTHR11588//PTHR11588:SF101 - TUBULIN // TUBULIN ALPHA-2 CHAIN-RELATED	0.00097	0.047507	3.054349
evm.model.AsparagusV1_03.2054	(1 of 1) PTHR14326//PTHR14326:SF18 - TARGETING PROTEIN FOR XKLP2 // SUBFAMILY NOT NAMED	0.00098	0.047507	11.28643
evm.model.AsparagusV1_01.2974	(1 of 2) PTHR33146:SF2 - ENDONUCLEASE 2	0.000989	0.047507	2.737939
evm.model.AsparagusV1_Unassigned.1059	(1 of 471) PF13041 - PPR repeat family (PPR_2)	0.000997	0.047507	2.249601
evm.model.AsparagusV1_05.2890	(1 of 1) K03189 - urease accessory protein (ureG)	0.001002	0.047507	5.464145
evm.model.AsparagusV1_07.2414	Missing annotation	0.001003	0.047507	0.047901
evm.model.AsparagusV1_Unassigned.1035	(1 of 1) PTHR10438:SF229 - THIOREDOXIN-LIKE 4, CHLOROPLASTIC	0.001012	0.047507	1.437807
evm.model.AsparagusV1_03.39	(1 of 3) K02915 - large subunit ribosomal protein L34e (RP-L34e, RPL34)	0.00102	0.047507	2.890034
evm.model.AsparagusV1_04.723	(1 of 3) PTHR33057//PTHR33057:SF1 - FAMILY NOT NAMED // OVATE FAMILY PROTEIN 13	0.001036	0.047507	3.234524
evm.model.AsparagusV1_08.447	(1 of 11) PF00314 - Thaumatin family (Thaumatin)	0.001041	0.047507	0.004248
evm.model.AsparagusV1_02.1346	Missing annotation	0.001045	0.047507	0.009546
evm.model.AsparagusV1_06.157	Missing annotation	0.001052	0.047507	2.524594
evm.model.AsparagusV1_03.1140	(1 of 1) PTHR31251:SF9 - SQUAMOSA PROMOTER-BINDING-LIKE PROTEIN 4-RELATED	0.001059	0.047507	25.19036
evm.model.AsparagusV1_03.1061	Missing annotation	0.001077	0.047507	8.786836
evm.model.AsparagusV1_03.357	(1 of 4) K18810 - cyclin D1/2/4, plant (CYCD1_2_4)	0.001078	0.047507	4.156411

evm.model.AsparagusV1_02.529	(1 of 1) PTHR11527:SF135 - 22.0 KDA HEAT SHOCK PROTEIN	0.001083	0.047507	2.4187
evm.model.AsparagusV1_08.3035	(1 of 2) PTHR24322//PTHR24322:SF619 - FAMILY NOT NAMED // 3-OXOACYL-[ACYL-CARRIER-PROTEIN] REDUCTASE, CHLOROPLASTIC	0.001084	0.047507	0.328934
evm.model.AsparagusV1_02.773	(1 of 3) PTHR11638//PTHR11638:SF69 - ATP-DEPENDENT CLP PROTEASE // SUBFAMILY NOT NAMED	0.001095	0.047507	2.153303
evm.model.AsparagusV1_03.1712	(1 of 3) PTHR24326:SF176 - HOMEOBOX-LEUCINE ZIPPER PROTEIN ATHB-13-RELATED	0.001097	0.047507	0.323977
evm.model.AsparagusV1_08.1554	Missing annotation	0.001101	0.047507	1.155497
evm.model.AsparagusV1_02.19	(1 of 1) PF07014 - Hs1pro-1 protein C-terminus (Hs1pro-1_C)	0.001102	0.047507	0.255306
evm.model.AsparagusV1_09.251	Missing annotation	0.001104	0.047507	1.225464
evm.model.AsparagusV1_03.2572	(1 of 2) PTHR24349//PTHR24349:SF115 - SERINE/THREONINE-PROTEIN KINASE // SUBFAMILY NOT NAMED	0.001116	0.047507	2.816692
evm.model.AsparagusV1_08.762	(1 of 2) PTHR20902//PTHR20902:SF0 - 41-2 PROTEIN ANTIGEN-RELATED // TRAFFICKING PROTEIN PARTICLE COMPLEX SUBUNIT 5	0.001122	0.047507	8.270756
evm.model.AsparagusV1_01.3625	(1 of 3) PTHR23515:SF3 - HIGH AFFINITY NITRATE TRANSPORTER 2.5	0.001123	0.047507	1.007282
evm.model.AsparagusV1_03.1770	(1 of 477) PF01535 - PPR repeat (PPR)	0.001124	0.047507	1.038843
evm.model.AsparagusV1_08.158	(1 of 2) K11826 - AP-2 complex subunit mu-1 (AP2M1)	0.001127	0.047507	0.002512
evm.model.AsparagusV1_04.1700	(1 of 33) PF04043 - Plant invertase/pectin methylesterase inhibitor (PMEI)	0.001128	0.047507	0.005527

evm.model.AsparagusV1_09.580	(1 of 1) PTHR21397:SF4 - ER MEMBRANE PROTEIN COMPLEX SUBUNIT 10	0.001129	0.047507	2.03901
evm.model.AsparagusV1_08.1885	Missing annotation	0.001132	0.047507	1.281368
evm.model.AsparagusV1_04.1070	(1 of 1) PTHR22814:SF155 - HEAVY METAL-ASSOCIATED ISOPRENYLATED PLANT PROTEIN 26	0.00114	0.047507	1.989206
evm.model.AsparagusV1_07.157	Missing annotation	0.001155	0.047507	1.414947
evm.model.AsparagusV1_04.1524	(1 of 3) K00031 - isocitrate dehydrogenase (IDH1, IDH2, icd)	0.001157	0.047507	6.723261
evm.model.AsparagusV1_03.2345	(1 of 1) PTHR22950//PTHR22950:SF288 - AMINO ACID TRANSPORTER // SUBFAMILY NOT NAMED	0.001168	0.047507	0.003452
evm.model.AsparagusV1_03.1790	(1 of 1) KOG2049 - Translational repressor MPT5/PUF4 and related RNA-binding proteins (Puf superfamily)	0.001168	0.047507	18.49304
evm.model.AsparagusV1_05.3034	(1 of 3) PTHR31949:SF3 - PROTEIN, PUTATIVE-RELATED	0.001179	0.047507	1.38327
evm.model.AsparagusV1_10.1390	(1 of 2) PTHR31052:SF2 - COBRA-LIKE PROTEIN 10-RELATED	0.001196	0.047507	0.002805
evm.model.AsparagusV1_09.226	(1 of 3) K05933 - aminocyclopropanecarboxylate oxidase (E1.14.17.4)	0.001198	0.047507	3.16563
evm.model.AsparagusV1_06.1061	(1 of 4) PTHR31169//PTHR31169:SF1 - FAMILY NOT NAMED // ZINC-FINGER DOMAIN OF MONOAMINE-OXIDASE A REPRESSOR R1 PROTEIN	0.001203	0.047507	6.356539
evm.model.AsparagusV1_01.3074	(1 of 1) PTHR22835:SF281 - SGNH HYDROLASE-TYPE ESTERASE SUPERFAMILY PROTEIN	0.001225	0.047507	2.058194
evm.model.AsparagusV1_04.147	(1 of 96) PF02365 - No apical meristem (NAM) protein (NAM)	0.001237	0.047507	89.93184

evm.model.AsparagusV1_01.3394	(1 of 1) PTHR13200//PTHR13200:SF0 - UNCHARACTERIZED // N(6)-ADENINE-SPECIFIC DNA METHYLTRANSFERASE 2	0.001241	0.047507	5.246238
evm.model.AsparagusV1_Unassigned.173	(1 of 1) K00225 - L-galactono-1,4-lactone dehydrogenase (GLDH)	0.001249	0.047507	1.888904
evm.model.AsparagusV1_08.2346	(1 of 9) K02183 - calmodulin (CALM)	0.001253	0.047507	0.092547
evm.model.AsparagusV1_04.2504	(1 of 5) PTHR23115//PTHR23115:SF157 - TRANSLATION FACTOR // SUBFAMILY NOT NAMED	0.001255	0.047507	4.67451
evm.model.AsparagusV1_05.2754	(1 of 1) K02212 - DNA replication licensing factor MCM4 (MCM4, CDC54)	0.00126	0.047507	12.82202
evm.model.AsparagusV1_03.3069	(1 of 2) PTHR12081//PTHR12081:SF46 - TRANSCRIPTION FACTOR E2F // E2F TRANSCRIPTION FACTOR-LIKE E2FE	0.001263	0.047507	2.051115
evm.model.AsparagusV1_05.3396	(1 of 1) PTHR23091//PTHR23091:SF195 - N-TERMINAL ACETYLTRANSFERASE // SUBFAMILY NOT NAMED	0.001272	0.047539	1.255192
evm.model.AsparagusV1_08.3048	(1 of 1) PF15477 - Small acidic protein family (SMAP)	0.001282	0.047543	2.050464
evm.model.AsparagusV1_02.150	(1 of 1) PTHR19375:SF164 - HEAT SHOCK 70 KDA PROTEIN 3	0.001331	0.047774	1.840777
evm.model.AsparagusV1_08.3606	(1 of 1) KOG4757 - Predicted telomere binding protein	0.001332	0.047774	3.54097
evm.model.AsparagusV1_07.375	(1 of 1) PTHR12802:SF52 - PROTEIN REVEILLE 4-RELATED	0.001332	0.047774	0.001543
evm.model.AsparagusV1_07.709	(1 of 4) PTHR24282//PTHR24282:SF11 - CYTOCHROME P450 FAMILY MEMBER // SUBFAMILY NOT NAMED	0.001347	0.047774	0.595537
evm.model.AsparagusV1_08.2718	(1 of 44) PF01535//PF13041//PF13812 - PPR repeat (PPR) // PPR repeat family (PPR_2) // Pentatricopeptide repeat domain (PPR_3)	0.001351	0.047774	7.756724

evm.model.AsparagusV1_07.1132	(1 of 4) PTHR22891//PTHR22891:SF20 - EUKARYOTIC TRANSLATION INITIATION FACTOR 2C // PROTEIN ARGONAUTE 4-RELATED	0.001356	0.047774	10.69801
evm.model.AsparagusV1_04.2863	(1 of 5) K01183 - chitinase (E3.2.1.14)	0.001368	0.047774	0.346884
evm.model.AsparagusV1_04.824	(1 of 3) PTHR22849//PTHR22849:SF31 - WDSAM1 PROTEIN // SUBFAMILY NOT NAMED	0.001388	0.047774	6.926477
evm.model.AsparagusV1_04.951	(1 of 2) PTHR11260:SF220 - GST-CONTAINING FLYWCH ZINC-FINGER PROTEIN	0.001399	0.047774	3.533794
evm.model.AsparagusV1_07.3519	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	0.001403	0.047774	1.939829
evm.model.AsparagusV1_02.2067	(1 of 2) PTHR11524//PTHR11524:SF11 - 60S RIBOSOMAL PROTEIN L7 // 60S RIBOSOMAL PROTEIN L7-1	0.00141	0.047774	3.04566
evm.model.AsparagusV1_07.426	(1 of 1) 3.5.4.17 - Adenosine-phosphate deaminase	0.00141	0.047774	3.087502
evm.model.AsparagusV1_01.2846	(1 of 16) 3.1.4.46 - Glycerophosphodiester phosphodiesterase / Glycerophosphoryl diester phosphodiesterase	0.001412	0.047774	1.459858
evm.model.AsparagusV1_01.3232	Missing annotation	0.001416	0.047774	1.028752
evm.model.AsparagusV1_07.173	Missing annotation	0.001422	0.047774	0.419945
evm.model.AsparagusV1_04.2924	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	0.001425	0.047774	1.967622
evm.model.AsparagusV1_08.1024	Missing annotation	0.001441	0.047774	7.520325
evm.model.AsparagusV1_02.927	(1 of 17) 3.4.22.33 - Fruit bromelain	0.00145	0.047774	0.145096
evm.model.AsparagusV1_04.242	Missing annotation	0.001458	0.047774	1.020446
evm.model.AsparagusV1_08.3444	(1 of 2) PTHR24115//PTHR24115:SF515 - FAMILY NOT NAMED // KINESIN MOTOR PROTEIN-RELATED PROTEIN-RELATED	0.001463	0.047774	1.05819

evm.model.AsparagusV1_08.2416	Missing annotation	0.00147	0.047774	1.617829
evm.model.AsparagusV1_06.817	(1 of 7) K01728 - pectate lyase (pel)	0.001482	0.04791	0.003446
evm.model.AsparagusV1_04.624	(1 of 1) PTHR31602:SF12 - GROWTH-REGULATING FACTOR 3-RELATED	0.001496	0.047942	2.088066
evm.model.AsparagusV1_01.2296	(1 of 7) PF00069//PF00582 - Protein kinase domain (Pkinase) // Universal stress protein family (Usp)	0.00153	0.048119	0.001761
evm.model.AsparagusV1_08.2629	(1 of 1) PTHR31865:SF3 - F22O13.28	0.001536	0.048119	0.004721
evm.model.AsparagusV1_Unassigned.1005	Missing annotation	0.001538	0.048119	0.15939
evm.model.AsparagusV1_10.1514	(1 of 19) PF00560//PF13855 - Leucine Rich Repeat (LRR_1) // Leucine rich repeat (LRR_8)	0.001541	0.048119	0.712994
evm.model.AsparagusV1_07.320	(1 of 3) PTHR27003:SF94 - RECEPTOR-LIKE PROTEIN KINASE HERK 1	0.001552	0.048244	0.249872
evm.model.AsparagusV1_07.1759	(1 of 1) PTHR13718//PTHR13718:SF63 - RIBOSOMAL S SUBUNIT // SUBFAMILY NOT NAMED	0.00157	0.048244	5.174294
evm.model.AsparagusV1_08.1703	Missing annotation	0.001592	0.048244	8.495011
evm.model.AsparagusV1_10.1668	(1 of 2) 1.18.1.2//1.6.99.1 - Ferredoxin--NADP(+) reductase // NADPH dehydrogenase / NADPH diaphorase	0.001592	0.048244	6.698989
evm.model.AsparagusV1_04.2486	(1 of 1) PTHR11661//PTHR11661:SF7 - 60S RIBOSOMAL PROTEIN L12 // 50S RIBOSOMAL PROTEIN L11, CHLOROPLASTIC-RELATED	0.001593	0.048244	1.935939
evm.model.AsparagusV1_05.2801	(1 of 1) PTHR24361:SF334 - MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 19-RELATED	0.001594	0.048244	3.453458
evm.model.AsparagusV1_04.1913	(1 of 2) PTHR31052:SF2 - COBRA-LIKE PROTEIN 10-RELATED	0.001597	0.048244	0.00454
evm.model.AsparagusV1_03.2694	(1 of 57) 3.2.1.39 - Glucan endo-1,3-beta-D-glucosidase / Laminarinase	0.0016	0.048244	0.404655

evm.model.AsparagusV1_03.1220	(1 of 2) PTHR31421:SF0 - PROTEIN BASIC PENTACYSTEINE1-RELATED	0.001605	0.048244	3.274473
evm.model.AsparagusV1_Unassigned.1046	(1 of 6) 2.4.1.185 - Flavanone 7-O-beta-glucosyltransferase	0.001608	0.048244	11.14257
evm.model.AsparagusV1_05.266	Missing annotation	0.001626	0.048244	6.164074
evm.model.AsparagusV1_05.3413	Missing annotation	0.001628	0.048244	0.173582
evm.model.AsparagusV1_04.2670	(1 of 1) PTHR31791//PTHR31791:SF8 - FAMILY NOT NAMED // ZIP4-LIKE PROTEIN	0.001636	0.048244	8.9823
evm.model.AsparagusV1_02.2163	(1 of 1) 2.7.1.56 - 1-phosphofructokinase / Fructose 1-phosphate kinase	0.001649	0.048244	1.075665
evm.model.AsparagusV1_08.563	(1 of 3) PTHR11527:SF169 - ALPHA-CRYSTALLIN DOMAIN OF HEAT SHOCK PROTEIN-CONTAINING PROTEIN	0.001673	0.048244	0.25074
evm.model.AsparagusV1_07.1945	(1 of 71) PF00168 - C2 domain (C2)	0.001678	0.048244	1.172964
evm.model.AsparagusV1_08.2993	(1 of 2) PTHR11772:SF18 - ALUMINUM INDUCED PROTEIN WITH YGL AND LRDR MOTIFS	0.001684	0.048244	0.165491
evm.model.AsparagusV1_07.3194	Missing annotation	0.001698	0.048244	0.019493
evm.model.AsparagusV1_03.1080	Missing annotation	0.001704	0.048244	16.68411
evm.model.AsparagusV1_03.1837	(1 of 3) PTHR31388//PTHR31388:SF2 - FAMILY NOT NAMED // PEROXIDASE 17	0.001705	0.048244	5.68804
evm.model.AsparagusV1_07.48	(1 of 1) PTHR13312//PTHR13312:SF2 - HIV-INDUCED PROTEIN-7-LIKE PROTEASE // SUBFAMILY NOT NAMED	0.001706	0.048244	0.344459
evm.model.AsparagusV1_06.1642	(1 of 1) PTHR10168:SF61 - F1N19.7	0.001709	0.048244	0.241353
evm.model.AsparagusV1_03.1998	(1 of 3) PTHR22953:SF22 - PURPLE ACID PHOSPHATASE 10	0.001739	0.048557	845.0669
evm.model.AsparagusV1_07.1129	Missing annotation	0.001749	0.048598	0.185826
evm.model.AsparagusV1_01.2107	Missing annotation	0.001776	0.048817	1.451587

evm.model.AsparagusV1_06.181	(1 of 2) K01961 - acetyl-CoA carboxylase, biotin carboxylase subunit (accC)	0.001778	0.048817	0.546289
evm.model.AsparagusV1_02.691	(1 of 5) PTHR22765//PTHR22765:SF46 - RING FINGER AND PROTEASE ASSOCIATED DOMAIN-CONTAINING // SUBFAMILY NOT NAMED	0.001778	0.048817	3.424904
evm.model.AsparagusV1_03.2449	(1 of 1) PTHR11751//PTHR11751:SF431 - SUBGROUP I AMINOTRANSFERASE RELATED // 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE 4-RELATED	0.001801	0.048817	1.192291
evm.model.AsparagusV1_07.139	(1 of 5) PTHR23056:SF45 - CALCINEURIN B-LIKE PROTEIN 4	0.001811	0.048817	1.015289
evm.model.AsparagusV1_06.1088	Missing annotation	0.001812	0.048817	0.013664
evm.model.AsparagusV1_04.317	(1 of 1) K12856 - pre-mRNA-processing factor 8 (PRPF8, PRP8)	0.001822	0.048856	3.678509
evm.model.AsparagusV1_07.617	(1 of 2) PTHR10555//PTHR10555:SF155 - SORTING NEXIN // SUBFAMILY NOT NAMED	0.001856	0.048865	2.436148
evm.model.AsparagusV1_05.3101	(1 of 15) PF05678 - VQ motif (VQ)	0.001857	0.048865	14.88131
evm.model.AsparagusV1_04.1069	(1 of 1) PTHR10044//PTHR10044:SF113 - INHIBITOR OF APOPTOSIS // SUBFAMILY NOT NAMED	0.00186	0.048865	9.250741
evm.model.AsparagusV1_08.587	(1 of 19) PF08541 - 3-Oxoacyl-[acyl-carrier-protein (ACP)] synthase III C terminal (ACP_syn_III_C)	0.001865	0.048865	4.975763
evm.model.AsparagusV1_07.3232	(1 of 13) PF04720 - PDDEXK-like family of unknown function (PDDEXK_6)	0.001887	0.049042	3.028401
evm.model.AsparagusV1_01.2254	(1 of 2) PTHR31683//PTHR31683:SF7 - FAMILY NOT NAMED // PECTATE LYASE 19-RELATED	0.00189	0.049042	0.003942

evm.model.AsparagusV1_Unassigned.369	(1 of 1) PTHR27003//PTHR27003:SF51 - FAMILY NOT NAMED // LEUCINE-RICH REPEAT-CONTAINING PROTEIN	0.001905	0.049042	0.004481
evm.model.AsparagusV1_08.2367	(1 of 4) K18810 - cyclin D1/2/4, plant (CYCD1_2_4)	0.001918	0.049042	5.718857
evm.model.AsparagusV1_03.2165	(1 of 2) PTHR23201//PTHR23201:SF10 - EXTENSIN, PROLINE-RICH PROTEIN // GIBBERELLIN-REGULATED GASA/GAST/SNAKIN FAMILY PROTEIN-RELATED	0.00192	0.049042	0.093668
evm.model.AsparagusV1_06.546	(1 of 2) PTHR21713 - NASCENT POLYPEPTIDE ASSOCIATED COMPLEX ALPHA SUBUNIT-RELATED	0.001955	0.04967	4.520315
evm.model.AsparagusV1_03.647	(1 of 1) PTHR33177:SF6 - F3O9.30	0.001961	0.04967	0.118617
evm.model.AsparagusV1_07.1773	(1 of 2) K03232 - elongation factor 1-beta (EEF1B)	0.001965	0.04967	3.064881
evm.model.AsparagusV1_06.389	(1 of 1) K09705 - hypothetical protein (K09705)	0.001966	0.04967	0.090661
evm.model.AsparagusV1_06.287	(1 of 1) PTHR31344//PTHR31344:SF1 - FAMILY NOT NAMED // F28J7.14 PROTEIN	0.001968	0.04967	3.832898
evm.model.AsparagusV1_06.585	(1 of 1) K03574 - 8-oxo-dGTP diphosphatase [EC:3.6.1.55] (mutT, NUDT15, MTH2)	0.00199	0.049839	2.487469
evm.model.AsparagusV1_01.2371	Missing annotation	0.001993	0.049839	2.969241

Table 8. List of differentially expressed genes with gene models in the current annotation for genes that originally did not have a gene model listed. Gene models listed had a 10% overlap. Significantly differentially expressed genes identified with a p-value < 0.5 and a false discovery rate (q-value) < 0.5 were only seen in the comparison of small and large male flower buds.

Gene name	Gene function	pval	qval	fc
evm.TU.AsparagusV1_Unassigned.228.V1.1	(1 of 2) K01052 - lysosomal acid lipase/cholesteryl ester hydrolase (LIPA)	1.06E-06	0.022199	1.652519
evm.TU.AsparagusV1_02.1925.V1.1	(1 of 3) PTHR11926:SF176 - UDP-GLYCOSYLTRANSFERASE 87A2	4.26E-06	0.02233	0.149047
evm.TU.AsparagusV1_05.3100.V1.1	(1 of 15) PF05678 - VQ motif (VQ)	2.19E-06	0.02233	1.069453
evm.TU.AsparagusV1_05.2586.V1.1	(1 of 1) PTHR32191//PTHR32191:SF17 - FAMILY NOT NAMED // TETRASPANIN-14-RELATED	8.37E-06	0.025062	0.145879
evm.TU.AsparagusV1_06.366.V1.1	(1 of 2) PTHR10288//PTHR10288:SF137 - KH DOMAIN CONTAINING RNA BINDING PROTEIN // SUBFAMILY NOT NAMED	8.09E-06	0.025062	2.807074
evm.TU.AsparagusV1_01.2003.V1.1	(1 of 3) PTHR11815:SF1 - SUCCINYL-COA LIGASE [ADP-FORMING] SUBUNIT BETA, MITOCHONDRIAL	8.35E-05	0.039005	1.152836
evm.TU.AsparagusV1_01.3072.V1.1	(1 of 1) K17413 - small subunit ribosomal protein S35 (MRPS35)	0.000138	0.039005	2.921975
evm.TU.AsparagusV1_01.3242.V1.1	(1 of 3) K15730 - cytosolic prostaglandin-E synthase (PTGES3)	2.53E-05	0.039005	2.47905
evm.TU.AsparagusV1_01.3451.V1.1	(1 of 1) PTHR12818//PTHR12818:SF0 - UNCHARACTERIZED // NEF-ASSOCIATED PROTEIN 1	5.84E-05	0.039005	1.280849
evm.TU.AsparagusV1_02.1180.V1.1	(1 of 1) K01733 - threonine synthase (thrC)	4.76E-05	0.039005	2.516489

evm.TU.AsparagusV1_02.645.V1.1	(1 of 4) PTHR13856//PTHR13856:SF95 - VHS DOMAIN CONTAINING PROTEIN FAMILY // F24J8.3 PROTEIN	0.000101	0.039005	0.070642
evm.TU.AsparagusV1_03.141.V1.1 or evm.TU.AsparagusV1_03.142.V1.1 or evm.TU.AsparagusV1_03.143.V1.1	(1 of 1) K14298 - mRNA export factor (RAE1, GLE2) or (1 of 20) PF14510 - ABC-transporter extracellular N-terminal (ABC_trans_N) or (1 of 4) PTHR10971//PTHR10971:SF16 - MRNA EXPORT FACTOR AND BUB3 // SUBFAMILY NOT NAMED	6.81E-05	0.039005	15.29097
evm.TU.AsparagusV1_03.245.V1.1	(1 of 1) K03252 - translation initiation factor 3 subunit C (EIF3C)	3.92E-05	0.039005	2.343124
evm.TU.AsparagusV1_03.612.V1.1	(1 of 1) PTHR11934//PTHR11934:SF8 - RIBOSE-5-PHOSPHATE ISOMERASE // RIBOSE-5-PHOSPHATE ISOMERASE 4, CHLOROPLASTIC-RELATED	0.00013	0.039005	14.79744
evm.TU.AsparagusV1_03.95.V1.1	Missing annotation	0.000108	0.039005	0.052682
evm.TU.AsparagusV1_04.1944.V1.1	(1 of 1) PTHR22942//PTHR22942:SF1 - RECA/RAD51/RADA DNA STRAND-PAIRING FAMILY MEMBER // MITOCHONDRIAL DNA REPAIR PROTEIN RECA HOMOLOG	8.13E-05	0.039005	6.568048
evm.TU.AsparagusV1_04.2061.V1.1	(1 of 1) PTHR24115//PTHR24115:SF413 - FAMILY NOT NAMED // KINESIN-LIKE PROTEIN KIN12A-RELATED	7.16E-05	0.039005	10.55817
evm.TU.AsparagusV1_04.2511.V1.1	(1 of 6) PF00560//PF07714//PF08263//PF13855 - Leucine Rich Repeat (LRR_1) // Protein tyrosine kinase (Pkinase_Tyr) // Leucine rich repeat N-terminal domain (LRRNT_2) // Leucine rich repeat (LRR_8)	0.000153	0.039005	96.24803
evm.TU.AsparagusV1_04.2683.V1.1	(1 of 1) PTHR14326//PTHR14326:SF15 - TARGETING PROTEIN FOR XKLP2 // CELL	3.47E-05	0.039005	10.67953

	CYCLE REGULATED MICROTUBULE ASSOCIATED PROTEIN			
evm.TU.AsparagusV1_04.411.V1.1	(1 of 1) K02328 - DNA polymerase delta subunit 2 (POLD2)	5.58E-05	0.039005	10.14724
evm.TU.AsparagusV1_04.473.V1.1	(1 of 2) KOG2846 - Predicted membrane protein	9.29E-05	0.039005	0.238247
evm.TU.AsparagusV1_04.80.V1.1	Missing annotation	9.27E-05	0.039005	11.67732
evm.TU.AsparagusV1_05.3043.V1.1 or evm.TU.AsparagusV1_05.3044.V1.1	(1 of 4) 3.2.1.15//3.2.1.67 - Polygalacturonase / Pectinase // Galacturan 1,4-alpha-galacturonidase / Poly(galacturonate) hydrolase or (1 of 41) 3.2.1.15 - Polygalacturonase / Pectinase	8.31E-05	0.039005	0.000266
evm.TU.AsparagusV1_05.3497.V1.1	(1 of 2) PTHR31251:SF4 - SQUAMOSA PROMOTER-BINDING-LIKE PROTEIN 8	5.14E-05	0.039005	95.6969
evm.TU.AsparagusV1_06.257.V1.1	Missing annotation	0.000103	0.039005	2.875107
evm.TU.AsparagusV1_07.1347.V1.1	(1 of 3) PTHR19321:SF7 - 65-KDA MICROTUBULE-ASSOCIATED PROTEIN 3-RELATED	0.000121	0.039005	10.62338
evm.TU.AsparagusV1_07.1547.V1.1	(1 of 1) K12662 - U4/U6 small nuclear ribonucleoprotein PRP4 (PRPF4, PRP4)	0.00013	0.039005	2.707068
evm.TU.AsparagusV1_07.1804.V1.1	(1 of 1) PTHR24031:SF228 - PROTEIN Y54G11A.3	0.000145	0.039005	5.191396
evm.TU.AsparagusV1_07.3481.V1.1	(1 of 2) PTHR10476//PTHR10476:SF8 - CHARGED MULTIVESICULAR BODY PROTEIN // VACUOLAR PROTEIN SORTING-ASSOCIATED PROTEIN 2 HOMOLOG 2	0.000103	0.039005	27.67007
evm.TU.AsparagusV1_07.3526.V1.1	(1 of 1) PTHR36027//PTHR36027:SF1 - FAMILY NOT NAMED // ASYNAPTIC 3	1.51E-05	0.039005	13.98209
evm.TU.AsparagusV1_07.579.V1.1	(1 of 1) PF00240//PF13855 - Ubiquitin family (ubiquitin) // Leucine rich repeat (LRR_8)	0.0001	0.039005	2.357893
evm.TU.AsparagusV1_07.846.V1.1	(1 of 5) PF01535//PF12854//PF13041//PF14432 - PPR repeat (PPR) // PPR repeat (PPR_1) // PPR	0.000133	0.039005	3.61609

	repeat family (PPR_2) // DYW family of nucleic acid deaminases (DYW_deaminase)			
evm.TU.AsparagusV1_08.1738.V1.1	(1 of 15) PF16135 - TPL-binding domain in jasmonate signalling (Jas)	5.81E-05	0.039005	62.29037
evm.TU.AsparagusV1_08.176.V1.1	(1 of 5) PTHR11566//PTHR11566:SF80 - DYNAMIN // DYNAMIN-RELATED PROTEIN 1C	0.0001	0.039005	29.84623
evm.TU.AsparagusV1_08.2837.V1.1	(1 of 1) K03109 - signal recognition particle subunit SRP9 (SRP9)	5.65E-05	0.039005	3.283961
evm.TU.AsparagusV1_08.2906.V1.1	(1 of 1) PTHR11614//PTHR11614:SF97 - PHOSPHOLIPASE-RELATED // SUBFAMILY NOT NAMED	8.78E-05	0.039005	0.03193
evm.TU.AsparagusV1_08.3225.V1.1	Missing annotation	2.85E-05	0.039005	4.42463
evm.TU.AsparagusV1_08.3226.V1.1	(1 of 1) PTHR11426:SF46 - HISTONE H3-LIKE CENTROMERIC PROTEIN A	6.20E-05	0.039005	8.954465
evm.TU.AsparagusV1_08.334.V1.1	(1 of 4) PTHR24078:SF280 - MYB TRANSCRIPTION FACTOR	0.000146	0.039005	2.501189
evm.TU.AsparagusV1_08.3514.V1.1	(1 of 3) 2.4.1.117 - Dolichyl-phosphate beta-glucosyltransferase	0.000125	0.039005	2.437584
evm.TU.AsparagusV1_08.474.V1.1	(1 of 2) K06944 - uncharacterized protein (K06944)	0.000115	0.039005	1.536642
evm.TU.AsparagusV1_08.645.V1.1	(1 of 1) 2.7.1.31 - Glycerate 3-kinase	4.62E-05	0.039005	2.740664
evm.TU.AsparagusV1_09.29.V1.1	(1 of 4) KOG2816 - Predicted transporter ADD1 (major facilitator superfamily)	5.83E-05	0.039005	2.821113
evm.TU.AsparagusV1_Unassigned.1172.V1.1	(1 of 3) PTHR19443:SF5 - HEXOKINASE-LIKE 1 PROTEIN	0.000115	0.039005	10.38967
evm.TU.AsparagusV1_Unassigned.687.V1.1	(1 of 2) PTHR12917//PTHR12917:SF18 - ASPARTYL PROTEASE DDI-RELATED // RHOMBOID DOMAIN-CONTAINING PROTEIN 3	6.32E-05	0.039005	1.446869

evm.TU.AsparagusV1_01.2629.V1.1	(1 of 45) PF05922 - Peptidase inhibitor I9 (Inhibitor_I9)	0.000159	0.039451	15.26829
evm.TU.AsparagusV1_01.3554.V1.1	(1 of 2) K01876 - aspartyl-tRNA synthetase (DARS, aspS)	0.000161	0.039451	2.69391
evm.TU.AsparagusV1_07.3858.V1.1	(1 of 3) PF14380 - Wall-associated receptor kinase C-terminal (WAK_assoc)	0.000164	0.039451	0.344341
evm.TU.AsparagusV1_08.102.V1.1	Missing annotation	0.000163	0.039451	26.97806
evm.TU.AsparagusV1_10.1523.V1.1	(1 of 1) K14795 - ribosomal RNA-processing protein 36 (RRP36)	0.000157	0.039451	2.737241
evm.TU.AsparagusV1_01.2614.V1.1	(1 of 3) PTHR23326//PTHR23326:SF1 - CCR4 NOT-RELATED // CCR4-NOT TRANSCRIPTION COMPLEX SUBUNIT 3	0.000196	0.039551	3.58082
evm.TU.AsparagusV1_03.2366.V1.1	(1 of 1) K18328 - lariat debranching enzyme [EC:3.1.-.-] (DBR1)	0.000188	0.039551	3.536658
evm.TU.AsparagusV1_04.689.V1.1	Missing annotation	0.000178	0.039551	13.66275
evm.TU.AsparagusV1_05.50.V1.1	(1 of 1) PTHR11413:SF56 - CYSTEINE PROTEINASE INHIBITOR 3-RELATED	0.000191	0.039551	0.215682
evm.TU.AsparagusV1_07.1195.V1.1	(1 of 1) PTHR30115:SF11 - NITROGEN REGULATORY PROTEIN P-II HOMOLOG	0.000192	0.039551	1.909343
evm.TU.AsparagusV1_07.502.V1.1	(1 of 2) PTHR11802//PTHR11802:SF71 - SERINE PROTEASE FAMILY S10 SERINE CARBOXYPEPTIDASE // SERINE CARBOXYPEPTIDASE-LIKE 26-RELATED	0.000193	0.039551	0.07044
evm.TU.AsparagusV1_Unassigned.503.V1.1	Missing annotation	0.000177	0.039551	0.390739
evm.TU.AsparagusV1_03.1497.V1.1	(1 of 1) PTHR18937//PTHR18937:SF222 - STRUCTURAL MAINTENANCE OF CHROMOSOMES SMC FAMILY MEMBER // SUBFAMILY NOT NAMED	0.000203	0.039551	25.92351
evm.TU.AsparagusV1_04.178.V1.1	(1 of 2) PTHR22884//PTHR22884:SF366 - SET DOMAIN PROTEINS // SUBFAMILY NOT NAMED	0.000202	0.039551	5.786686

evm.TU.AsparagusV1_02.167.V1.1	(1 of 2) K12881 - THO complex subunit 4 (THOC4, ALY)	0.000211	0.040163	5.246706
evm.TU.AsparagusV1_03.2908.V1.1	(1 of 1) 1.14.11.4 - Procollagen-lysine 5-dioxygenase / Procollagen-lysine,2-oxoglutarate 5-dioxygenase	0.000216	0.040163	3.795628
evm.TU.AsparagusV1_06.660.V1.1	(1 of 2) PF12090 - Spt20 family (Spt20)	0.00021	0.040163	5.237999
evm.TU.AsparagusV1_07.3250.V1.1	(1 of 3) PTHR12446//PTHR12446:SF21 - TESMIN/TSO1-RELATED // CRC DOMAIN-CONTAINING PROTEIN TSO1-RELATED	0.000216	0.040163	4.160565
evm.TU.AsparagusV1_01.2644.V1.1	(1 of 1) PTHR33322:SF4 - BAG FAMILY MOLECULAR CHAPERONE REGULATOR 6	0.000225	0.040432	14.30105
evm.TU.AsparagusV1_03.2973.V1.1	(1 of 2) PTHR32295//PTHR32295:SF14 - FAMILY NOT NAMED // PROTEIN IQ-DOMAIN 31-RELATED	0.000235	0.040432	2.144203
evm.TU.AsparagusV1_04.2141.V1.1	(1 of 1) PTHR24115//PTHR24115:SF480 - FAMILY NOT NAMED // ATP BINDING MICROTUBULE MOTOR FAMILY PROTEIN	0.000229	0.040432	23.33006
evm.TU.AsparagusV1_08.1178.V1.1	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	0.000237	0.040432	4.534577
evm.TU.AsparagusV1_10.1812.V1.1	(1 of 4) PF04842 - Plant protein of unknown function (DUF639) (DUF639)	0.000231	0.040432	0.43785
evm.TU.AsparagusV1_01.2429.V1.1	(1 of 1) PTHR11006//PTHR11006:SF68 - PROTEIN ARGININE N-METHYLTRANSFERASE // PROTEIN ARGININE N-METHYLTRANSFERASE PRMT10	0.000272	0.041607	6.602436
evm.TU.AsparagusV1_01.3304.V1.1	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	0.000274	0.041607	4.788251
evm.TU.AsparagusV1_02.639.V1.1	(1 of 2) PTHR22849//PTHR22849:SF34 - WDSAM1 PROTEIN // SUBFAMILY NOT NAMED	0.000259	0.041607	0.071271

evm.TU.AsparagusV1_02.970.V1.1	(1 of 3) PTHR10250//PTHR10250:SF16 - MICROSOMAL GLUTATHIONE S-TRANSFERASE // GLUTATHIONE S-TRANSFERASE-RELATED	0.000278	0.041607	2.643546
evm.TU.AsparagusV1_05.31.V1.1	(1 of 2) K11518 - mitochondrial import receptor subunit TOM40 (TOM40)	0.000254	0.041607	10.31478
evm.TU.AsparagusV1_06.205.V1.1	(1 of 1) PTHR24423//PTHR24423:SF514 - TWO-COMPONENT SENSOR HISTIDINE KINASE // HISTIDINE KINASE CKI1	0.000253	0.041607	6.340079
evm.TU.AsparagusV1_08.1214.V1.1	(1 of 1) PTHR23083//PTHR23083:SF431 - TETRATRICOPEPTIDE REPEAT PROTEIN, TPR // SUBFAMILY NOT NAMED	0.000269	0.041607	9.435095
evm.TU.AsparagusV1_08.1457.V1.1	(1 of 2) K11430 - histone-lysine N-methyltransferase EZH2 (EZH2)	0.000256	0.041607	4.056083
evm.TU.AsparagusV1_08.584.V1.1	(1 of 2) K02945 - small subunit ribosomal protein S1 (RP-S1, rpsA)	0.000248	0.041607	2.095493
evm.TU.AsparagusV1_08.951.V1.1	(1 of 1) PTHR10992//PTHR10992:SF773 - ALPHA/BETA HYDROLASE FOLD-CONTAINING PROTEIN // PROTEIN AUXIN RESPONSE 4	0.00028	0.041607	1.967289
evm.TU.AsparagusV1_03.213.V1.1	(1 of 2) PTHR31132//PTHR31132:SF2 - FAMILY NOT NAMED // EXPRESSED PROTEIN	0.000285	0.041629	21.05023
evm.TU.AsparagusV1_04.511.V1.1	(1 of 49) 3.6.3.44 - Xenobiotic-transporting ATPase / Steroid-transporting ATPase	0.000287	0.041629	10.07919
evm.TU.AsparagusV1_02.1234.V1.1	(1 of 2) PTHR22811//PTHR22811:SF68 - TRANSMEMBRANE EMP24 DOMAIN-CONTAINING PROTEIN // SUBFAMILY NOT NAMED	0.000294	0.041923	0.518123
evm.TU.AsparagusV1_03.2504.V1.1	Missing annotation	0.000298	0.041923	2.052917

evm.TU.AsparagusV1_06.238.V1.1	(1 of 2) K10405 - kinesin family member C1 (KIFC1)	0.000298	0.041923	7.966555
evm.TU.AsparagusV1_08.3152.V1.1	(1 of 1) PTHR22814//PTHR22814:SF133 - COPPER TRANSPORT PROTEIN ATOX1-RELATED // SUBFAMILY NOT NAMED	0.000297	0.041923	0.306694
evm.TU.AsparagusV1_01.2127.V1.1 or evm.TU.AsparagusV1_01.2129.V1.1	(1 of 2) K04485 - DNA repair protein RadA/Sms (sms, radA)	0.000341	0.041992	9.664006
evm.TU.AsparagusV1_01.2281.V1.1	(1 of 1) K15264 - putative methyltransferase [EC:2.1.1.-] (NSUN5, WBSCR20)	0.000352	0.041992	5.903841
evm.TU.AsparagusV1_01.3325.V1.1	(1 of 1) K12626 - U6 snRNA-associated Sm-like protein LSm7 (LSM7)	0.000357	0.041992	2.511017
evm.TU.AsparagusV1_01.3463.V1.1 or evm.TU.AsparagusV1_01.3464.V1.1	(1 of 1) 3.4.19.11 - Gamma-D-glutamyl-meso-diaminopimelate peptidase / Gamma-D-glutamyl-meso-diaminopimelate peptidase I or Missing annotation	0.000317	0.041992	12.02848
evm.TU.AsparagusV1_02.1608.V1.1	(1 of 1) PTHR13734//PTHR13734:SF47 - TRNA-NUCLEOTIDYLTRANSFERASE/POLY A POLYMERASE FAMILY MEMBER // POLYNUCLEOTIDE ADENYLYLTRANSFERASE DOMAIN AND RNA RECOGNITION MOTIF-CONTAINING PROTEIN-RELATED	0.000333	0.041992	4.157294
evm.TU.AsparagusV1_02.1904.V1.1	(1 of 4) PTHR19306//PTHR19306:SF1 - STRUCTURAL MAINTENANCE OF CHROMOSOMES 5,6 SMC5, SMC6 // STRUCTURAL MAINTENANCE OF CHROMOSOMES PROTEIN 5	0.000382	0.041992	2.655549
evm.TU.AsparagusV1_02.2090.V1.1	(1 of 1) PTHR23244//PTHR23244:SF315 - KELCH REPEAT DOMAIN // SUBFAMILY NOT NAMED	0.000326	0.041992	98.07103

evm.TU.AsparagusV1_03.1256.V1.1 or evm.TU.AsparagusV1_03.1257.V1.1	(1 of 1) PTHR11732//PTHR11732:SF237 - ALDO/KETO REDUCTASE // SUBFAMILY NOT NAMED or (1 of 4) PTHR11732:SF239 - ALDO/KETO REDUCTASE-RELATED	0.000335	0.041992	0.00265
evm.TU.AsparagusV1_03.240.V1.1	(1 of 38) PF03080 - Domain of unknown function (DUF239) (DUF239)	0.000328	0.041992	1.48438
evm.TU.AsparagusV1_04.364.V1.1	(1 of 2) PTHR31384//PTHR31384:SF19 - FAMILY NOT NAMED // AUXIN RESPONSE FACTOR 17	0.000349	0.041992	5.265378
evm.TU.AsparagusV1_04.829.V1.1	(1 of 2) PTHR23201:SF16 - PROLINE-RICH PROTEIN 4	0.000341	0.041992	0.07789
evm.TU.AsparagusV1_05.234.V1.1	Missing annotation	0.000352	0.041992	3.245542
evm.TU.AsparagusV1_05.2491.V1.1	(1 of 1) PTHR32467:SF23 - AP2-LIKE ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR AIL5	0.000365	0.041992	5.417923
evm.TU.AsparagusV1_06.105.V1.1	(1 of 1) PTHR32278//PTHR32278:SF8 - FAMILY NOT NAMED // PHLOEM PROTEIN 2-A10-RELATED	0.00039	0.041992	0.02125
evm.TU.AsparagusV1_06.71.V1.1 or evm.TU.AsparagusV1_06.75.V1.1 or evm.TU.AsparagusV1_06.76.V1.1 or evm.TU.AsparagusV1_06.77.V1.1	(1 of 7) PTHR10809//PTHR10809:SF50 - VESICLE-ASSOCIATED MEMBRANE PROTEIN-ASSOCIATED PROTEIN // VESICLE-ASSOCIATED PROTEIN 1-1-RELATED or Missing annotation or (1 of 1) PTHR10209//PTHR10209:SF159 - OXIDOREDUCTASE, 2OG-FE II OXYGENASE FAMILY PROTEIN // SUBFAMILY NOT NAMED or (1 of 7) PTHR10809//PTHR10809:SF50 - VESICLE-ASSOCIATED MEMBRANE PROTEIN-ASSOCIATED PROTEIN // VESICLE-ASSOCIATED PROTEIN 1-1-RELATED	0.000322	0.041992	70.03198

evm.TU.AsparagusV1_07.1715.V1.1	(1 of 1) PTHR33882//PTHR33882:SF3 - FAMILY NOT NAMED // RPM1-INTERACTING PROTEIN 4 (RIN4) FAMILY PROTEIN	0.00037	0.041992	0.00171
evm.TU.AsparagusV1_07.1840.V1.1	(1 of 1) PTHR11476//PTHR11476:SF5 - HISTIDYL-TRNA SYNTHETASE // CLASS II AARS AND BIOTIN SYNTHETASES SUPERFAMILY PROTEIN-RELATED	0.000383	0.041992	1.766219
evm.TU.AsparagusV1_07.1935.V1.1 or evm.TU.AsparagusV1_07.1936.V1.1	(1 of 4) PTHR27000//PTHR27000:SF99 - FAMILY NOT NAMED // LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE PXL1	0.000386	0.041992	0.176963
evm.TU.AsparagusV1_07.3179.V1.1	(1 of 1) K14552 - NET1-associated nuclear protein 1 (U3 small nucleolar RNA-associated protein 17) (NAN1, UTP17, WDR75)	0.000392	0.041992	2.455612
evm.TU.AsparagusV1_07.337.V1.1	(1 of 214) PF01535//PF13041 - PPR repeat (PPR) // PPR repeat family (PPR_2)	0.000309	0.041992	2.718045
evm.TU.AsparagusV1_07.3890.V1.1 or evm.TU.AsparagusV1_07.3891.V1.1	Missing annotation or (1 of 2) K03113 - translation initiation factor 1 (EIF1, SUI1)	0.000308	0.041992	0.343747
evm.TU.AsparagusV1_07.59.V1.1	Missing annotation	0.000345	0.041992	398.4652
evm.TU.AsparagusV1_07.863.V1.1	(1 of 18) PF04782 - Protein of unknown function (DUF632) (DUF632)	0.000399	0.041992	3.451912
evm.TU.AsparagusV1_08.3192.V1.1	(1 of 20) PF05553 - Cotton fibre expressed protein (DUF761)	0.00038	0.041992	0.002772
evm.TU.AsparagusV1_08.3556.V1.1	(1 of 2) PTHR10811//PTHR10811:SF13 - FRINGE-RELATED // SUBFAMILY NOT NAMED	0.000361	0.041992	26.40669
evm.TU.AsparagusV1_09.654.V1.1	(1 of 1) 2.8.4.5 - tRNA (N(6)-L-threonylcarbamoyladenosine(37)-C(2))-methylthiotransferase / Methylthio-threonylcarbamoyl-adenosine transferase B	0.000382	0.041992	3.511646

evm.TU.AsparagusV1_10.1391.V1.1	(1 of 2) KOG0796 - Spliceosome subunit	0.000367	0.041992	2.333179
evm.TU.AsparagusV1_01.2523.V1.1	(1 of 1) K09832 - cytochrome P450, family 710, subfamily A (CYP710A)	0.000406	0.04228	0.003638
evm.TU.AsparagusV1_06.1953.V1.1	(1 of 1) PTHR11758:SF11 - 40S RIBOSOMAL PROTEIN S15A-2-RELATED	0.000418	0.042522	3.111359
evm.TU.AsparagusV1_08.276.V1.1	(1 of 1) PTHR11528//PTHR11528:SF53 - HEAT SHOCK PROTEIN 90 // HEAT SHOCK PROTEIN 90-1	0.000418	0.042522	462.7063
evm.TU.AsparagusV1_03.2601.V1.1	(1 of 37) PTHR11353 - CHAPERONIN	0.000428	0.043165	8.705347
evm.TU.AsparagusV1_08.2377.V1.1	(1 of 17) K15032 - mTERF domain-containing protein, mitochondrial (MTERFD)	0.000431	0.043185	1.801046
evm.TU.AsparagusV1_04.1902.V1.1 or evm.TU.AsparagusV1_04.1903.V1.1	(1 of 2) PTHR34124:SF2 - F16B3.27 PROTEIN or (1 of 1) K10750 - chromatin assembly factor 1 subunit A (CHAF1A)	0.00047	0.043821	9.010911
evm.TU.AsparagusV1_04.2206.V1.1	Missing annotation	0.000479	0.043821	9.187367
evm.TU.AsparagusV1_04.3107.V1.1	(1 of 1) PTHR11669//PTHR11669:SF5 - REPLICATION FACTOR C / DNA POLYMERASE III GAMMA-TAU SUBUNIT // REPLICATION FACTOR C SUBUNIT 2	0.000481	0.043821	4.926075
evm.TU.AsparagusV1_04.372.V1.1	Missing annotation	0.000454	0.043821	0.407154
evm.TU.AsparagusV1_04.376.V1.1	(1 of 1) PTHR10795//PTHR10795:SF394 - PROPROTEIN CONVERTASE SUBTILISIN/KEXIN // SUBFAMILY NOT NAMED	0.000453	0.043821	0.022461
evm.TU.AsparagusV1_05.181.V1.1	(1 of 477) PF01535 - PPR repeat (PPR)	0.00046	0.043821	2.858266
evm.TU.AsparagusV1_06.1714.V1.1	(1 of 6) PTHR24067:SF100 - UBIQUITIN-CONJUGATING ENZYME FAMILY PROTEIN-RELATED	0.000477	0.043821	2.741798
evm.TU.AsparagusV1_06.440.V1.1 or evm.TU.AsparagusV1_06.441.V1.1	(1 of 10) PF12776 - Myb/SANT-like DNA-binding domain (Myb_DNA-bind_3) or (1 of 3) PTHR10553//PTHR10553:SF9 - SMALL	0.000445	0.043821	2.29804

	NUCLEAR RIBONUCLEOPROTEIN // SUBFAMILY NOT NAMED			
evm.TU.AsparagusV1_08.83.V1.1	(1 of 3) PF16029 - Domain of unknown function (DUF4787) (DUF4787)	0.000444	0.043821	0.0056
evm.TU.AsparagusV1_Unassigned.264.V1.1	(1 of 2) PTHR27000//PTHR27000:SF84 - FAMILY NOT NAMED // LRR RECEPTOR-LIKE SERINE/THREONINE-PROTEIN KINASE ERL1-RELATED	0.000475	0.043821	6.398599
evm.TU.AsparagusV1_01.2082.V1.1	(1 of 4) PTHR12299//PTHR12299:SF27 - HYALURONIC ACID-BINDING PROTEIN 4 // SUBFAMILY NOT NAMED	0.00051	0.044453	3.852375
evm.TU.AsparagusV1_04.1770.V1.1	(1 of 1) K03136 - transcription initiation factor TFIIE subunit alpha (TFIIE1, GTF2E1, TFA1, tfe)	0.000504	0.044453	2.799231
evm.TU.AsparagusV1_05.2880.V1.1	(1 of 2) PTHR10052//PTHR10052:SF9 - 60S RIBOSOMAL PROTEIN L18A // SUBFAMILY NOT NAMED	0.000507	0.044453	0.399959
evm.TU.AsparagusV1_07.3167.V1.1	(1 of 1) PTHR12687:SF8 - NOC2P FAMILY PROTEIN	0.000505	0.044453	104.119
evm.TU.AsparagusV1_07.3830.V1.1	Missing annotation	0.000503	0.044453	0.75282
evm.TU.AsparagusV1_09.269.V1.1 or evm.TU.AsparagusV1_09.270.V1.1	(1 of 1) PTHR31346:SF5 - DAG-LIKE PROTEIN or (1 of 1) 2.5.1.3//2.7.1.49 - Thiamine-phosphate diphosphorylase / TMP pyrophosphorylase // Hydroxymethylpyrimidine kinase	0.000493	0.044453	4.502005
evm.TU.AsparagusV1_Unassigned.488.V1.1	Missing annotation	0.000509	0.044453	19.5325
evm.TU.AsparagusV1_02.412.V1.1	(1 of 3) PTHR31683//PTHR31683:SF8 - FAMILY NOT NAMED // MAJOR POLLEN ALLERGEN-LIKE PROTEIN	0.000521	0.044705	0.006087

evm.TU.AsparagusV1_06.11.V1.1	(1 of 8) PF04782//PF04783 - Protein of unknown function (DUF632) (DUF632) // Protein of unknown function (DUF630) (DUF630)	0.000539	0.044705	4.208665
evm.TU.AsparagusV1_07.44.V1.1	(1 of 1) K12599 - antiviral helicase SKI2 (SKI2, SKIV2L)	0.000536	0.044705	4.209421
evm.TU.AsparagusV1_07.806.V1.1	(1 of 2) PTHR10641:SF473 - MYB TRANSCRIPTION FACTOR	0.000536	0.044705	18.59565
evm.TU.AsparagusV1_07.925.V1.1	(1 of 4) PF15365 - Proline-rich nuclear receptor coactivator (PNRC)	0.000524	0.044705	0.061009
evm.TU.AsparagusV1_08.1199.V1.1	(1 of 6) PF00235 - Profilin (Profilin)	0.000516	0.044705	7.460876
evm.TU.AsparagusV1_03.397.V1.1	(1 of 2) PF13359 - DDE superfamily endonuclease (DDE_Tnp_4)	0.00055	0.044824	2.858655
evm.TU.AsparagusV1_07.1742.V1.1	(1 of 9) PF01535//PF13812 - PPR repeat (PPR) // Pentatricopeptide repeat domain (PPR_3)	0.000553	0.044934	4.716291
evm.TU.AsparagusV1_01.2695.V1.1 or evm.TU.AsparagusV1_01.2696.V1.1	Missing annotation or (1 of 1) K14791 - periodic tryptophan protein 1 (PWP1)	0.000578	0.044974	2.433282
evm.TU.AsparagusV1_03.1221.V1.1	(1 of 1) PTHR24326//PTHR24326:SF236 - FAMILY NOT NAMED // HOMEOBOX-LEUCINE ZIPPER PROTEIN HDG12	0.000586	0.044974	3.91658
evm.TU.AsparagusV1_03.189.V1.1	Missing annotation	0.000574	0.044974	7.134963
evm.TU.AsparagusV1_03.2690.V1.1 or evm.TU.AsparagusV1_03.2691.V1.1	Missing annotation or (1 of 1) K14309 - nuclear pore complex protein Nup93 (NUP93, NIC96)	0.000565	0.044974	5.174748
evm.TU.AsparagusV1_03.3024.V1.1	(1 of 1) PTHR31407//PTHR31407:SF7 - FAMILY NOT NAMED // PSBP DOMAIN-CONTAINING PROTEIN 5, CHLOROPLASTIC	0.000574	0.044974	9.086673
evm.TU.AsparagusV1_03.775.V1.1	Missing annotation	0.000569	0.044974	24.45369
evm.TU.AsparagusV1_04.3163.V1.1	(1 of 1) PTHR11070//PTHR11070:SF15 - UVRD / RECB / PCRA DNA HELICASE FAMILY MEMBER // SUBFAMILY NOT NAMED	0.00059	0.044974	5.792609

evm.TU.AsparagusV1_07.816.V1.1	(1 of 1) PTHR10641//PTHR10641:SF645 - MYB-LIKE DNA-BINDING PROTEIN MYB // MYB DOMAIN PROTEIN 110	0.000563	0.044974	22.99322
evm.TU.AsparagusV1_Unassigned.1119.V1.1	(1 of 1) PTHR22880//PTHR22880:SF174 - FALZ-RELATED BROMODOMAIN-CONTAINING PROTEINS // DNA-BINDING BROMODOMAIN-CONTAINING PROTEIN	0.000571	0.044974	2.590415
evm.TU.AsparagusV1_Unassigned.379.V1.1	(1 of 1) PTHR23050//PTHR23050:SF167 - CALCIUM BINDING PROTEIN // SUBFAMILY NOT NAMED	0.00058	0.044974	1.554903
evm.TU.AsparagusV1_01.2461.V1.1	(1 of 1) K14310 - nuclear pore complex protein Nup205 (NUP205, NUP192)	0.000625	0.045119	6.843288
evm.TU.AsparagusV1_02.1780.V1.1	Missing annotation	0.000604	0.045119	0.04892
evm.TU.AsparagusV1_04.1842.V1.1	(1 of 2) PTHR13286//PTHR13286:SF8 - SAP30 // SUBFAMILY NOT NAMED	0.000613	0.045119	10.87951
evm.TU.AsparagusV1_04.2078.V1.1	(1 of 5) PTHR22835:SF184 - GDSL ESTERASE/LIPASE EXL1-RELATED	0.000604	0.045119	0.004204
evm.TU.AsparagusV1_04.918.V1.1	(1 of 1) K03164 - DNA topoisomerase II [EC:5.99.1.3] (TOP2)	0.000622	0.045119	16.73187
evm.TU.AsparagusV1_06.1213.V1.1 or evm.TU.AsparagusV1_06.1214.V1.1	(1 of 1) K09493 - T-complex protein 1 subunit alpha (CCT1, TCP1) or (1 of 37) PTHR11353 - CHAPERONIN	0.000629	0.045119	4.862835
evm.TU.AsparagusV1_06.456.V1.1	(1 of 1) K03267 - peptide chain release factor subunit 3 (ERF3, GSPT)	0.000628	0.045119	1.976612
evm.TU.AsparagusV1_07.2589.V1.1	(1 of 3) PTHR13832:SF314 - PROTEIN PHOSPHATASE 2C 10-RELATED	0.000627	0.045119	0.232983
evm.TU.AsparagusV1_09.643.V1.1	(1 of 1) K02327 - DNA polymerase delta subunit 1 (POLD1)	0.000604	0.045119	5.711496
evm.TU.AsparagusV1_02.2125.V1.1	(1 of 8) PTHR10353//PTHR10353:SF29 - GLYCOSYL HYDROLASE // BETA-GLUCOSIDASE 1-RELATED	0.00066	0.045988	15.73602

evm.TU.AsparagusV1_08.1561.V1.1 or evm.TU.AsparagusV1_08.1562.V1.1	(1 of 3) 3.2.1.50 - Alpha-N-acetylglucosaminidase / NAG	0.000664	0.045988	4.466383
evm.TU.AsparagusV1_03.2857.V1.1	(1 of 2) PTHR10288//PTHR10288:SF155 - KH DOMAIN CONTAINING RNA BINDING PROTEIN // SUBFAMILY NOT NAMED	0.000674	0.046125	5.015012
evm.TU.AsparagusV1_04.3579.V1.1	(1 of 2) PTHR24115//PTHR24115:SF405 - FAMILY NOT NAMED // ATP BINDING MICROTUBULE MOTOR FAMILY PROTEIN	0.000685	0.046224	2.189013
evm.TU.AsparagusV1_03.495.V1.1	(1 of 2) PTHR10071//PTHR10071:SF161 - TRANSCRIPTION FACTOR GATA GATA BINDING FACTOR // SUBFAMILY NOT NAMED	0.000709	0.046461	13.17922
evm.TU.AsparagusV1_04.2892.V1.1	(1 of 1) PF15072 - Domain of unknown function (DUF4539) (DUF4539)	0.00071	0.046461	12.82187
evm.TU.AsparagusV1_06.242.V1.1	(1 of 1) PTHR10366//PTHR10366:SF350 - NAD DEPENDENT EPIMERASE/DEHYDRATASE // SUBFAMILY NOT NAMED	0.000709	0.046461	0.208035
evm.TU.AsparagusV1_07.1488.V1.1	(1 of 72) 3.1.1.11 - Pectinesterase / Pectin methylesterase	0.000714	0.046461	0.004092
evm.TU.AsparagusV1_07.886.V1.1 or evm.TU.AsparagusV1_07.887.V1.1	(1 of 4) PTHR24031:SF292 - ATP-DEPENDENT RNA HELICASE DDX54 or (1 of 3) PF08147 - DBP10CT (NUC160) domain (DBP10CT)	0.000713	0.046461	3.446123
evm.TU.AsparagusV1_08.400.V1.1	(1 of 11) 2.7.11.1//2.7.11.26 - Non-specific serine/threonine protein kinase / Threonine-specific protein kinase // [Tau protein] kinase / TTK	0.000705	0.046461	0.006266
evm.TU.AsparagusV1_08.1122.V1.1	Missing annotation	0.000727	0.0466	4.369647
evm.TU.AsparagusV1_Unassigned.868.V1.1	(1 of 1) K05302 - SET domain-containing protein 6 (SETD6)	0.000724	0.0466	2.21205

evm.TU.AsparagusV1_01.2363.V1.1	(1 of 1) PTHR12789 - DENSITY-REGULATED PROTEIN HOMOLOG	0.000924	0.047507	1.640687
evm.TU.AsparagusV1_01.2600.V1.1	Missing annotation	0.000852	0.047507	133.8598
evm.TU.AsparagusV1_01.2737.V1.1	(1 of 1) PTHR12565//PTHR12565:SF149 - STEROL REGULATORY ELEMENT-BINDING PROTEIN // SUBFAMILY NOT NAMED	0.001074	0.047507	0.09531
evm.TU.AsparagusV1_01.3192.V1.1	(1 of 1) K02606 - origin recognition complex subunit 4 (ORC4)	0.001196	0.047507	7.820859
evm.TU.AsparagusV1_02.121.V1.1	(1 of 1) K07025//K18551 - putative hydrolase of the HAD superfamily (K07025) // pyrimidine and pyridine-specific 5'-nucleotidase (SDT1)	0.000934	0.047507	0.457464
evm.TU.AsparagusV1_02.2007.V1.1	(1 of 1) PTHR36359:SF1 - RESISTANCE TO PHYTOPHTHORA 1 PROTEIN	0.001046	0.047507	6.40162
evm.TU.AsparagusV1_02.2080.V1.1	(1 of 1) K11654 - SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 [EC:3.6.4.-] (SMARCA5, SNF2H, ISWI)	0.000798	0.047507	36.80527
evm.TU.AsparagusV1_02.2191.V1.1 or evm.TU.AsparagusV1_02.2192.V1.1	Missing annotations	0.000772	0.047507	0.002598
evm.TU.AsparagusV1_02.221.V1.1	(1 of 1) K16546 - FGFR1 oncogene partner (FGFR10P)	0.001252	0.047507	37.80239
evm.TU.AsparagusV1_02.225.V1.1	(1 of 1) PF00069//PF12819//PF13855 - Protein kinase domain (Pkinase) // Carbohydrate-binding protein of the ER (Malectin_like) // Leucine rich repeat (LRR_8)	0.000839	0.047507	2.870828
evm.TU.AsparagusV1_02.277.V1.1	(1 of 3) PTHR24098//PTHR24098:SF4 - FAMILY NOT NAMED // ACYL-COA N-ACYLTRANSFERASE WITH RING/FYVE/PHD-TYPE ZINC FINGER DOMAIN-RELATED	0.001104	0.047507	5.646885

evm.TU.AsparagusV1_02.510.V1.1	(1 of 2) PTHR22792//PTHR22792:SF52 - LUPUS LA PROTEIN-RELATED // LA-RELATED PROTEIN 6B	0.000983	0.047507	6.850568
evm.TU.AsparagusV1_02.515.V1.1	(1 of 1) K03575 - A/G-specific adenine glycosylase (mutY)	0.001005	0.047507	5.21888
evm.TU.AsparagusV1_02.534.V1.1	(1 of 1) K01800 - maleylacetoacetate isomerase (maiA, GSTZ1)	0.000897	0.047507	0.293403
evm.TU.AsparagusV1_03.1207.V1.1	(1 of 7) PF08879//PF08880 - WRC (WRC) // QLQ (QLQ)	0.001099	0.047507	13.17254
evm.TU.AsparagusV1_03.1394.V1.1	(1 of 1) PF03763//PF08423 - Remorin, C-terminal region (Remorin_C) // Rad51 (Rad51)	0.000762	0.047507	5.975909
evm.TU.AsparagusV1_03.154.V1.1 or evm.TU.AsparagusV1_03.155.V1.1	Missing annotations	0.001034	0.047507	2.483654
evm.TU.AsparagusV1_03.1603.V1.1	(1 of 2) PTHR31062:SF44 - XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE PROTEIN 27-RELATED	0.000816	0.047507	0.115729
evm.TU.AsparagusV1_03.1611.V1.1	(1 of 16) PF03080//PF14365 - Domain of unknown function (DUF239) (DUF239) // Domain of unknown function (DUF4409) (DUF4409)	0.001036	0.047507	0.27519
evm.TU.AsparagusV1_03.1997.V1.1	(1 of 2) PTHR36737//PTHR36737:SF1 - FAMILY NOT NAMED // EXPRESSED PROTEIN	0.000817	0.047507	2.930708
evm.TU.AsparagusV1_03.2693.V1.1	(1 of 1) PTHR24089:SF370 - MITOCHONDRIAL SUBSTRATE CARRIER FAMILY PROTEIN	0.00099	0.047507	6.612801
evm.TU.AsparagusV1_03.460.V1.1	(1 of 37) PF02298 - Plastocyanin-like domain (Cu_bind_like)	0.001027	0.047507	0.400107
evm.TU.AsparagusV1_03.532.V1.1 or evm.TU.AsparagusV1_03.533.V1.1	(1 of 2) 5.4.99.27 - tRNA pseudouridine(13) synthase / tRNA Psi(13) synthase or (1 of 1)	0.001026	0.047507	2.977456

	K06176 - tRNA pseudouridine13 synthase [EC:5.4.99.27] (truD, PUS7)			
evm.TU.AsparagusV1_03.589.V1.1	(1 of 2) PTHR10694:SF41 - JUMONJI DOMAIN-CONTAINING PROTEIN 18-RELATED	0.001091	0.047507	0.195583
evm.TU.AsparagusV1_04.1381.V1.1	(1 of 2) PTHR32116:SF3 - GALACTURONOSYLTRANSFERASE 3-RELATED	0.001081	0.047507	27.03692
evm.TU.AsparagusV1_04.1396.V1.1 or evm.TU.AsparagusV1_04.1397.V1.1	(1 of 3) PTHR24326:SF267 - HOMEOBOX-LEUCINE ZIPPER PROTEIN HDG4-RELATED	0.00083	0.047507	3.699661
evm.TU.AsparagusV1_04.1530.V1.1	(1 of 2) PF11595 - Protein of unknown function (DUF3245) (DUF3245)	0.000761	0.047507	2.351105
evm.TU.AsparagusV1_04.1664.V1.1	Missing annotation	0.000957	0.047507	0.013395
evm.TU.AsparagusV1_04.1898.V1.1	(1 of 2) PTHR11524//PTHR11524:SF11 - 60S RIBOSOMAL PROTEIN L7 // 60S RIBOSOMAL PROTEIN L7-1	0.001056	0.047507	3.425201
evm.TU.AsparagusV1_04.1950.V1.1	(1 of 1) PTHR11206//PTHR11206:SF88 - MULTIDRUG RESISTANCE PROTEIN // MATE EFFLUX FAMILY PROTEIN	0.001004	0.047507	0.503354
evm.TU.AsparagusV1_04.2359.V1.1	(1 of 6) PTHR11260//PTHR11260:SF287 - GLUTATHIONE S-TRANSFERASE, GST, SUPERFAMILY, GST DOMAIN CONTAINING // SUBFAMILY NOT NAMED	0.000867	0.047507	0.009456
evm.TU.AsparagusV1_04.3265.V1.1	(1 of 1) K12180 - COP9 signalosome complex subunit 7 (COPS7, CSN7)	0.001216	0.047507	2.674536
evm.TU.AsparagusV1_04.373.V1.1 or evm.TU.AsparagusV1_04.374.V1.1	Missing annotation or (1 of 232) PF00076 - RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain) (RRM_1)	0.000823	0.047507	1.698833

evm.TU.AsparagusV1_04.51.V1.1	(1 of 1) PTHR10910//PTHR10910:SF94 - EUKARYOTE SPECIFIC DSRNA BINDING PROTEIN // SUBFAMILY NOT NAMED	0.001219	0.047507	1.821547
evm.TU.AsparagusV1_04.830.V1.1	(1 of 2) PTHR23201:SF16 - PROLINE-RICH PROTEIN 4	0.001193	0.047507	0.009055
evm.TU.AsparagusV1_05.2587.V1.1	(1 of 19) PF07714//PF08263//PF13855 - Protein tyrosine kinase (Pkinase_Tyr) // Leucine rich repeat N-terminal domain (LRRNT_2) // Leucine rich repeat (LRR_8)	0.000971	0.047507	9.173798
evm.TU.AsparagusV1_05.2661.V1.1	(1 of 1) PTHR32227:SF88 - GLUCAN ENDO-1,3-BETA-GLUCOSIDASE 12-RELATED	0.000753	0.047507	8.997479
evm.TU.AsparagusV1_05.2927.V1.1	(1 of 3) PTHR16254:SF30 - K(+) EFFLUX ANTIPORTER 4	0.000753	0.047507	2.707664
evm.TU.AsparagusV1_05.2988.V1.1	(1 of 2) K16810 - TBCC domain-containing protein 1 (TBCCD1)	0.00091	0.047507	1.915159
evm.TU.AsparagusV1_05.3180.V1.1	(1 of 16) PF05641 - Agenet domain (Agenet)	0.000848	0.047507	2.333194
evm.TU.AsparagusV1_05.3183.V1.1	(1 of 4) K10956 - protein transport protein SEC61 subunit alpha (SEC61A)	0.000965	0.047507	5.046962
evm.TU.AsparagusV1_05.51.V1.1	(1 of 1) 2.7.1.148//2.7.4.26 - 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase / CMK // Isopentenyl phosphate kinase	0.000935	0.047507	2.235369
evm.TU.AsparagusV1_05.54.V1.1	(1 of 2) K03232 - elongation factor 1-beta (EEF1B)	0.001214	0.047507	4.162864
evm.TU.AsparagusV1_06.1015.V1.1	(1 of 1) PF11317 - Protein of unknown function (DUF3119) (DUF3119)	0.000873	0.047507	0.009209
evm.TU.AsparagusV1_06.1017.V1.1	(1 of 2) PTHR31235//PTHR31235:SF51 - FAMILY NOT NAMED // PEROXIDASE 25	0.001111	0.047507	2.144256
evm.TU.AsparagusV1_06.1165.V1.1	(1 of 2) PTHR11071//PTHR11071:SF291 - PEPTIDYL-PROLYL CIS-TRANS ISOMERASE // SUBFAMILY NOT NAMED	0.001129	0.047507	2.042036

evm.TU.AsparagusV1_06.1194.V1.1	(1 of 3) K13199 - plasminogen activator inhibitor 1 RNA-binding protein (SERBP1)	0.001238	0.047507	2.466164
evm.TU.AsparagusV1_06.1952.V1.1	Missing annotation	0.001188	0.047507	2.517723
evm.TU.AsparagusV1_06.273.V1.1	Missing annotation	0.001251	0.047507	5.276758
evm.TU.AsparagusV1_06.276.V1.1	Missing annotation	0.001098	0.047507	15.83286
evm.TU.AsparagusV1_06.676.V1.1	(1 of 2) K10753 - histone chaperone ASF1 (ASF1)	0.000753	0.047507	11.30639
evm.TU.AsparagusV1_06.724.V1.1	(1 of 1) PTHR13832:SF218 - PROTEIN PHOSPHATASE 2C 57	0.000983	0.047507	6.379511
evm.TU.AsparagusV1_06.791.V1.1	(1 of 2) PTHR21677:SF3 - TSL-KINASE INTERACTING PROTEIN 1	0.000875	0.047507	34.37305
evm.TU.AsparagusV1_07.1117.V1.1	Missing annotation	0.001043	0.047507	46.11656
evm.TU.AsparagusV1_07.1280.V1.1	(1 of 1) K06228 - fused (FU)	0.001202	0.047507	8.039569
evm.TU.AsparagusV1_07.1464.V1.1	(1 of 2) 2.1.1.220 - tRNA (adenine(58)-N(1))-methyltransferase / tRNA m(1)A(58) methyltransferase	0.000856	0.047507	2.882505
evm.TU.AsparagusV1_07.1477.V1.1	(1 of 93) PTHR24078 - DNAJ HOMOLOG SUBFAMILY C MEMBER	0.000929	0.047507	10.22037
evm.TU.AsparagusV1_07.1491.V1.1	(1 of 3) PTHR31384//PTHR31384:SF8 - FAMILY NOT NAMED // AUXIN RESPONSE FACTOR 2	0.001108	0.047507	64.43871
evm.TU.AsparagusV1_07.1536.V1.1	(1 of 2) PTHR11353//PTHR11353:SF23 - CHAPERONIN // T-COMPLEX PROTEIN 1 SUBUNIT BETA	0.000988	0.047507	6.612557
evm.TU.AsparagusV1_07.1703.V1.1	(1 of 1) 1.14.13.81 - Magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase / Mg-protoporphyrin IX monomethyl ester oxidative cyclase	0.000832	0.047507	0.009261
evm.TU.AsparagusV1_07.1867.V1.1	(1 of 1) KOG0106//KOG0130//KOG0533 - Alternative splicing factor SRp55/B52/SRp75 (RRM superfamily) // RNA-binding protein	0.001228	0.047507	2.460118

	RBM8/Tsunagi (RRM superfamily) // RRM motif-containing protein			
evm.TU.AsparagusV1_07.1944.V1.1	Missing annotation	0.001101	0.047507	0.002284
evm.TU.AsparagusV1_07.1972.V1.1	(1 of 1) PTHR22912:SF106 - GLUTATHIONE REDUCTASE, MITOCHONDRIAL	0.000864	0.047507	2.086043
evm.TU.AsparagusV1_07.2024.V1.1	(1 of 6) PF12043 - Domain of unknown function (DUF3527) (DUF3527)	0.00088	0.047507	0.207046
evm.TU.AsparagusV1_07.2081.V1.1	(1 of 3) PTHR12313//PTHR12313:SF2 - RNF5 // E3 UBIQUITIN-PROTEIN LIGASE RMA3	0.001172	0.047507	1.574447
evm.TU.AsparagusV1_07.2101.V1.1	(1 of 2) PTHR14209:SF8 - GDSL ESTERASE/LIPASE CPRD49	0.00104	0.047507	0.420586
evm.TU.AsparagusV1_07.2103.V1.1 or evm.TU.AsparagusV1_07.2104.V1.1	(1 of 1) PTHR36055//PTHR36055:SF1 - FAMILY NOT NAMED // C2H2-LIKE ZINC FINGER PROTEIN or Missing annotation	0.000978	0.047507	0.02148
evm.TU.AsparagusV1_07.2403.V1.1	(1 of 2) K07088 - uncharacterized protein (K07088)	0.00086	0.047507	2.48446
evm.TU.AsparagusV1_07.2548.V1.1	(1 of 2) PF06916 - Protein of unknown function (DUF1279) (DUF1279)	0.000944	0.047507	5.640702
evm.TU.AsparagusV1_07.2739.V1.1	(1 of 4) PTHR15341//PTHR15341:SF3 - SUN-COR STEROID HORMONE RECEPTOR CO-REPRESSOR // NUCLEAR NUCLEIC ACID-BINDING PROTEIN C1D	0.000995	0.047507	2.485744
evm.TU.AsparagusV1_07.3501.V1.1	(1 of 2) K06944 - uncharacterized protein (K06944)	0.000791	0.047507	3.537742
evm.TU.AsparagusV1_07.3565.V1.1 or evm.TU.AsparagusV1_07.3566.V1.1	Missing annotation or (1 of 1) K01754 - threonine dehydratase (E4.3.1.19, ilvA, tdcB)	0.001107	0.047507	1.86426
evm.TU.AsparagusV1_07.3879.V1.1	(1 of 1) K01696 - tryptophan synthase beta chain (trpB)	0.000864	0.047507	3.316254
evm.TU.AsparagusV1_07.732.V1.1	(1 of 1) K03238 - translation initiation factor 2 subunit 2 (EIF2S2)	0.000789	0.047507	1.966097

evm.TU.AsparagusV1_08.1086.V1.1	(1 of 1) PTHR13068:SF13 - MITOCHONDRIAL TRANSCRIPTION TERMINATION FACTOR FAMILY PROTEIN	0.001087	0.047507	4.112294
evm.TU.AsparagusV1_08.1153.V1.1	(1 of 3) K12837 - splicing factor U2AF 65 kDa subunit (U2AF2)	0.001148	0.047507	1.829373
evm.TU.AsparagusV1_08.1364.V1.1	(1 of 5) PTHR23213//PTHR23213:SF210 - FORMIN-RELATED // FORMIN-LIKE PROTEIN 15A-RELATED	0.001118	0.047507	3.075904
evm.TU.AsparagusV1_08.192.V1.1	(1 of 2) PTHR11945:SF208 - MADS-BOX PROTEIN SOC1	0.000894	0.047507	2.665248
evm.TU.AsparagusV1_08.1966.V1.1	(1 of 1) PTHR31096:SF13 - ACT DOMAIN-CONTAINING PROTEIN 5	0.000964	0.047507	0.009562
evm.TU.AsparagusV1_08.2256.V1.1	(1 of 1) PTHR32297:SF1 - SODIUM CHANNEL MODIFIER 1	0.000764	0.047507	1.525391
evm.TU.AsparagusV1_08.2476.V1.1	Missing annotation	0.000809	0.047507	3.406489
evm.TU.AsparagusV1_08.2745.V1.1	(1 of 27) PF00295 - Glycosyl hydrolases family 28 (Glyco_hydro_28)	0.001042	0.047507	2.30727
evm.TU.AsparagusV1_08.2938.V1.1 or evm.TU.AsparagusV1_08.2939.V1.1 or evm.TU.AsparagusV1_08.2940.V1.1	(1 of 1) K03626 - nascent polypeptide-associated complex subunit alpha (EGD2, NACA) or Missing annotations	0.001028	0.047507	2.91049
evm.TU.AsparagusV1_08.2966.V1.1	Missing annotation	0.000902	0.047507	0.213506
evm.TU.AsparagusV1_08.3201.V1.1	(1 of 1) PTHR11073//PTHR11073:SF17 - CALRETICULIN AND CALNEXIN // SUBFAMILY NOT NAMED	0.001015	0.047507	6.086108
evm.TU.AsparagusV1_08.3224.V1.1	Missing annotation	0.001066	0.047507	0.003096
evm.TU.AsparagusV1_08.3273.V1.1	(1 of 3) K01885 - glutamyl-tRNA synthetase (EARS, gltX)	0.001242	0.047507	5.818132
evm.TU.AsparagusV1_08.3453.V1.1	(1 of 4) K12896 - splicing factor, arginine/serine-rich 7 (SFRS7)	0.001079	0.047507	2.392138
evm.TU.AsparagusV1_08.51.V1.1	Missing annotation	0.00115	0.047507	3.777287

evm.TU.AsparagusV1_08.67.V1.1	(1 of 42) PF00892 - EamA-like transporter family (EamA)	0.001248	0.047507	2.744636
evm.TU.AsparagusV1_08.801.V1.1	(1 of 4) PTHR27001:SF62 - PROTEIN KINASE FAMILY PROTEIN-RELATED	0.001217	0.047507	0.011069
evm.TU.AsparagusV1_09.165.V1.1	(1 of 1) PTHR14379//PTHR14379:SF6 - LIMKAIN B LKAP // EMB|CAB71880.1	0.001145	0.047507	8.649891
evm.TU.AsparagusV1_09.224.V1.1	(1 of 1) PTHR13561:SF20 - MUTAGEN-SENSITIVE 101	0.000848	0.047507	40.07572
evm.TU.AsparagusV1_09.248.V1.1	(1 of 44) PF01535//PF13041//PF13812 - PPR repeat (PPR) // PPR repeat family (PPR_2) // Pentatricopeptide repeat domain (PPR_3)	0.001254	0.047507	3.40365
evm.TU.AsparagusV1_09.479.V1.1	Missing annotation	0.001182	0.047507	0.071058
evm.TU.AsparagusV1_09.515.V1.1	(1 of 2) K17800 - LETM1 and EF-hand domain-containing protein 1, mitochondrial (LETM1, MDM38)	0.001267	0.047507	1.709681
evm.TU.AsparagusV1_10.1707.V1.1	(1 of 1) PTHR11260:SF166 - GLUTATHIONE S-TRANSFERASE D2-RELATED	0.001233	0.047507	9.730121
evm.TU.AsparagusV1_10.1788.V1.1	(1 of 66) PF01535//PF13041//PF14432 - PPR repeat (PPR) // PPR repeat family (PPR_2) // DYW family of nucleic acid deaminases (DYW_deaminase)	0.001243	0.047507	1.322443
evm.TU.AsparagusV1_Unassigned.1116.V1.1	(1 of 4) KOG0508//KOG4412 - Ankyrin repeat protein // 26S proteasome regulatory complex, subunit PSMD10	0.001069	0.047507	3.098345
evm.TU.AsparagusV1_Unassigned.407.V1.1	(1 of 1) K17776 - metaxin (MTX)	0.000944	0.047507	10.66583
evm.TU.AsparagusV1_Unassigned.46.V1.1	(1 of 1) PTHR11739:SF4 - CITRATE SYNTHASE, PEROXISOMAL	0.001247	0.047507	0.309734
evm.TU.AsparagusV1_Unassigned.722.V1.1	(1 of 18) PF03763 - Remorin, C-terminal region (Remorin_C)	0.000915	0.047507	5.43445
evm.TU.AsparagusV1_Unassigned.787.V1.1	(1 of 1) K14842 - ribosome biogenesis protein NSA2 (NSA2)	0.000906	0.047507	3.788655

evm.TU.AsparagusV1_Unassigned.827.V1.1	(1 of 4) PF00076//PF00658 - RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain) (RRM_1) // Poly-adenylate binding protein, unique domain (PABP)	0.000774	0.047507	2.451037
evm.TU.AsparagusV1_07.1953.V1.1	(1 of 1) KOG1162 - Predicted small molecule transporter	0.001273	0.047539	0.134662
evm.TU.AsparagusV1_02.800.V1.1	(1 of 2) PTHR22952//PTHR22952:SF179 - CAMP-RESPONSE ELEMENT BINDING PROTEIN-RELATED // SUBFAMILY NOT NAMED	0.001284	0.047543	8.318767
evm.TU.AsparagusV1_04.1006.V1.1	Missing annotation	0.001283	0.047543	0.645461
evm.TU.AsparagusV1_06.190.V1.1	(1 of 1) PTHR34682:SF1 - AT HOOK MOTIF-CONTAINING PROTEIN	0.001283	0.047543	4.63442
evm.TU.AsparagusV1_07.3253.V1.1	(1 of 2) PTHR24362:SF255 - SERINE/THREONINE-PROTEIN KINASE NEK1	0.001293	0.047763	5.403685
evm.TU.AsparagusV1_01.2291.V1.1	(1 of 1) PTHR21483:SF18 - RNA POLYMERASE II-ASSOCIATED PROTEIN 1	0.001385	0.047774	3.225923
evm.TU.AsparagusV1_01.3096.V1.1	(1 of 1) K03251 - translation initiation factor 3 subunit D (EIF3D)	0.001436	0.047774	2.95457
evm.TU.AsparagusV1_01.3122.V1.1	(1 of 4) 1.14.13.104 - (+)-menthofuran synthase / Menthofuran synthase	0.001401	0.047774	0.280352
evm.TU.AsparagusV1_01.3312.V1.1	(1 of 3) K00208 - enoyl- (fabI)	0.001458	0.047774	0.090906
evm.TU.AsparagusV1_01.3370.V1.1	(1 of 2) PTHR13068:SF5 - MITOCHONDRIAL TRANSCRIPTION TERMINATION FACTOR FAMILY PROTEIN	0.001437	0.047774	2.418838
evm.TU.AsparagusV1_01.3564.V1.1	(1 of 1) K02516 - protein arginine N-methyltransferase 5 (PRMT5, HSL7)	0.001432	0.047774	32.18298
evm.TU.AsparagusV1_02.2175.V1.1	(1 of 1) PTHR11207//PTHR11207:SF1 - RIBONUCLEASE III // SUBFAMILY NOT NAMED	0.001357	0.047774	1.834477

evm.TU.AsparagusV1_02.2236.V1.1	(1 of 1) PTHR24012//PTHR24012:SF411 - FAMILY NOT NAMED // EXPRESSED PROTEIN	0.001301	0.047774	1.578747
evm.TU.AsparagusV1_02.811.V1.1 or evm.TU.AsparagusV1_02.812.V1.1	(1 of 6) 3.6.5.4 - Signal-recognition-particle GTPase or (1 of 1) KOG0780 - Signal recognition particle, subunit Srp54	0.001461	0.047774	30.61985
evm.TU.AsparagusV1_03.3137.V1.1	(1 of 1) PTHR22951//PTHR22951:SF29 - CLATHRIN ASSEMBLY PROTEIN // SUBFAMILY NOT NAMED	0.001383	0.047774	0.063011
evm.TU.AsparagusV1_03.808.V1.1	(1 of 2) PTHR12886//PTHR12886:SF0 - PIG-M MANNOSYLTRANSFERASE // GPI MANNOSYLTRANSFERASE 1	0.001305	0.047774	0.007117
evm.TU.AsparagusV1_04.1200.V1.1 or evm.TU.AsparagusV1_04.1201.V1.1	(1 of 3) PTHR31062//PTHR31062:SF16 - FAMILY NOT NAMED // XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE PROTEIN 29-RELATED	0.001411	0.047774	0.108387
evm.TU.AsparagusV1_04.1226.V1.1	(1 of 1) PTHR11599//PTHR11599:SF55 - PROTEASOME SUBUNIT ALPHA/BETA // SUBFAMILY NOT NAMED	0.001412	0.047774	1.756635
evm.TU.AsparagusV1_04.2179.V1.1	(1 of 2) PTHR33222//PTHR33222:SF9 - FAMILY NOT NAMED // PROTEIN CURVATURE THYLAKOID 1B, CHLOROPLASTIC	0.00132	0.047774	3.483401
evm.TU.AsparagusV1_04.3377.V1.1 or evm.TU.AsparagusV1_04.3378.V1.1	Missing annotation or (1 of 3) PTHR23213:SF190 - FORMIN-LIKE PROTEIN 17-RELATED	0.001302	0.047774	2.518977
evm.TU.AsparagusV1_04.752.V1.1	(1 of 3) PTHR22870:SF91 - REGULATOR OF CHROMOSOME CONDENSATION (RCC1) FAMILY WITH FYVE ZINC FINGER DOMAIN-RELATED	0.001371	0.047774	3.659178

evm.TU.AsparagusV1_05.348.V1.1	(1 of 2) PTHR23336//PTHR23336:SF10 - ZINC FINGER CW-TYPE COILED-COIL DOMAIN PROTEIN 3. // B3 DOMAIN-CONTAINING TRANSCRIPTION REPRESSOR VAL1-RELATED	0.001377	0.047774	1.408629
evm.TU.AsparagusV1_06.1095.V1.1	(1 of 2) K02355 - elongation factor G (fusA, GFM, EFG)	0.00147	0.047774	6.024096
evm.TU.AsparagusV1_06.1149.V1.1	(1 of 1) K06640 - serine/threonine-protein kinase ATR (ATR)	0.001341	0.047774	3.940907
evm.TU.AsparagusV1_06.1548.V1.1	(1 of 1) PF15375 - Domain of unknown function (DUF4602) (DUF4602)	0.001329	0.047774	2.874363
evm.TU.AsparagusV1_06.426.V1.1	(1 of 1) PTHR13140//PTHR13140:SF304 - MYOSIN // MYOSIN IA HEAVY CHAIN-RELATED	0.001409	0.047774	4.382689
evm.TU.AsparagusV1_07.1099.V1.1	(1 of 2) K02835 - peptide chain release factor 1 (prfA, MTRF1, MRF1)	0.001441	0.047774	3.821408
evm.TU.AsparagusV1_07.1589.V1.1	(1 of 1) PTHR23315//PTHR23315:SF133 - BETA CATENIN-RELATED ARMADILLO REPEAT-CONTAINING // SUBFAMILY NOT NAMED	0.001395	0.047774	0.180984
evm.TU.AsparagusV1_07.1907.V1.1	(1 of 1) K15711 - SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A3 [EC:3.6.4.- 6.3.2.19] (SMARCA3, HLTF)	0.001312	0.047774	3.302249
evm.TU.AsparagusV1_07.1996.V1.1	(1 of 477) PF01535 - PPR repeat (PPR)	0.001454	0.047774	2.251643
evm.TU.AsparagusV1_07.213.V1.1 or evm.TU.AsparagusV1_07.214.V1.1	(1 of 13) PTHR19375:SF232 - MEDIATOR OF RNA POLYMERASE II TRANSCRIPTION SUBUNIT 37E-RELATED	0.001436	0.047774	3.064906
evm.TU.AsparagusV1_07.277.V1.1	(1 of 1) PTHR24115//PTHR24115:SF525 - FAMILY NOT NAMED // ATP BINDING MICROTUBULE MOTOR FAMILY PROTEIN	0.001413	0.047774	5.856564

evm.TU.AsparagusV1_07.2936.V1.1	(1 of 13) PF04720 - PDDEXK-like family of unknown function (PDDEXK_6)	0.001322	0.047774	2.406295
evm.TU.AsparagusV1_07.3437.V1.1	(1 of 34) PF00651 - BTB/POZ domain (BTB)	0.001332	0.047774	2.796599
evm.TU.AsparagusV1_07.3717.V1.1	Missing annotation	0.001471	0.047774	3.821845
evm.TU.AsparagusV1_08.1303.V1.1	Missing annotation	0.001464	0.047774	1.733807
evm.TU.AsparagusV1_08.2521.V1.1	(1 of 3) PTHR31388//PTHR31388:SF2 - FAMILY NOT NAMED // PEROXIDASE 17	0.001377	0.047774	0.207809
evm.TU.AsparagusV1_08.2549.V1.1	(1 of 2) PTHR31235:SF69 - PEROXIDASE 42	0.001445	0.047774	0.070684
evm.TU.AsparagusV1_08.3260.V1.1	(1 of 3) K03264 - translation initiation factor 6 (EIF6)	0.001365	0.047774	253.8175
evm.TU.AsparagusV1_08.3432.V1.1	(1 of 1) K18732 - SAP domain-containing ribonucleoprotein (SARNP, CIP29, THO1)	0.001429	0.047774	2.775157
evm.TU.AsparagusV1_08.3630.V1.1	(1 of 2) PTHR22950//PTHR22950:SF256 - AMINO ACID TRANSPORTER // AUXIN TRANSPORTER PROTEIN 1-RELATED	0.001347	0.047774	0.132161
evm.TU.AsparagusV1_09.238.V1.1	(1 of 3) PTHR22950//PTHR22950:SF295 - AMINO ACID TRANSPORTER // AMINO ACID PERMEASE 2	0.00147	0.047774	0.071651
evm.TU.AsparagusV1_09.368.V1.1	(1 of 1) PTHR10388//PTHR10388:SF11 - EUKARYOTIC TRANSLATION INITIATION FACTOR SUI1 // APO PROTEIN 3, MITOCHONDRIAL	0.001449	0.047774	2.349073
evm.TU.AsparagusV1_Unassigned.411.V1.1	(1 of 1) K12874 - intron-binding protein aquarius (AQR)	0.001302	0.047774	1.030759
evm.TU.AsparagusV1_Unassigned.73.V1.1 or evm.TU.AsparagusV1_Unassigned.74.V1.1	(1 of 1) PTHR34449//PTHR34449:SF2 - FAMILY NOT NAMED // ATP BINDING / ATPASE-RELATED or (1 of 2) PTHR12383//PTHR12383:SF5 - PROTEASE FAMILY S26 MITOCHONDRIAL INNER MEMBRANE PROTEASE-RELATED //	0.001314	0.047774	2.967292

	PEPTIDASE-S24/S26 DOMAIN-CONTAINING PROTEIN			
evm.TU.AsparagusV1_03.2210.V1.1	(1 of 10) K10406 - kinesin family member C2/C3 (KIFC2_3)	0.001473	0.047787	6.954547
evm.TU.AsparagusV1_03.1158.V1.1	(1 of 2) K02178 - checkpoint serine/threonine-protein kinase (BUB1)	0.001479	0.047897	7.971072
evm.TU.AsparagusV1_01.2370.V1.1	(1 of 3) PF03208 - PRA1 family protein (PRA1)	0.001487	0.047942	0.53222
evm.TU.AsparagusV1_04.1359.V1.1	(1 of 1) K10782 - fatty acyl-ACP thioesterase A (FATA)	0.001493	0.047942	1.263756
evm.TU.AsparagusV1_05.11.V1.1	Missing annotation	0.001496	0.047942	2.946598
evm.TU.AsparagusV1_06.267.V1.1	(1 of 1) K11836 - ubiquitin carboxyl-terminal hydrolase 5/13 [EC:3.4.19.12] (USP5_13, UBP14)	0.001495	0.047942	6.178902
evm.TU.AsparagusV1_10.1766.V1.1	(1 of 3) PTHR31384:SF2 - AUXIN RESPONSE FACTOR 8	0.001488	0.047942	0.224186
evm.TU.AsparagusV1_04.1098.V1.1	(1 of 17) PF08879 - WRC (WRC)	0.001514	0.0481	10.24747
evm.TU.AsparagusV1_05.2670.V1.1	(1 of 1) PTHR27003//PTHR27003:SF44 - FAMILY NOT NAMED // LRR RECEPTOR-LIKE SERINE/THREONINE-PROTEIN KINASE MEE39-RELATED	0.001524	0.0481	14.50694
evm.TU.AsparagusV1_07.3255.V1.1 or evm.TU.AsparagusV1_07.3256.V1.1	(1 of 29) PF01535//PF12854//PF13041 - PPR repeat (PPR) // PPR repeat (PPR_1) // PPR repeat family (PPR_2) or Missing annotation	0.001524	0.0481	4.610029
evm.TU.AsparagusV1_07.3455.V1.1	(1 of 4) PTHR21717//PTHR21717:SF27 - TELOMERIC REPEAT BINDING PROTEIN // TELOMERE REPEAT-BINDING FACTOR 2-RELATED	0.001512	0.0481	4.095722
evm.TU.AsparagusV1_08.3130.V1.1	(1 of 3) PTHR11615//PTHR11615:SF150 - NITRATE, FROMATE, IRON DEHYDROGENASE // SUBFAMILY NOT NAMED	0.001506	0.0481	0.060539

evm.TU.AsparagusV1_08.3350.V1.1	(1 of 2) PTHR27000//PTHR27000:SF197 - FAMILY NOT NAMED // INFLORESCENCE AND ROOT APICES RECEPTOR-LIKE KINASE	0.001511	0.0481	9.834266
evm.TU.AsparagusV1_02.960.V1.1	(1 of 1) K03037 - 26S proteasome regulatory subunit N7 (PSMD6, RPN7)	0.00154	0.048119	2.10146
evm.TU.AsparagusV1_08.1088.V1.1	(1 of 4) K09377 - cysteine and glycine-rich protein (CSRP)	0.001539	0.048119	0.027041
evm.TU.AsparagusV1_03.34.V1.1	(1 of 1) K01551//K15015 - arsenite-transporting ATPase [EC:3.6.3.16] (arsA, ASNA1) // solute carrier family 32 (vesicular inhibitory amino acid transporter) (SLC32A, VGAT)	0.001544	0.048127	3.639492
evm.TU.AsparagusV1_01.2840.V1.1	(1 of 1) PTHR10535//PTHR10535:SF2 - DNA-DIRECTED RNA POLYMERASES I, II, AND III SUBUNIT RPABC1 // DNA-DIRECTED RNA POLYMERASE V SUBUNIT 5A-RELATED	0.001573	0.048244	3.54116
evm.TU.AsparagusV1_01.2933.V1.1 or evm.TU.AsparagusV1_01.2934.V1.1	(1 of 7) PTHR10366//PTHR10366:SF307 - NAD DEPENDENT EPIMERASE/DEHYDRATASE // TETRAKETIDE ALPHA-PYRONE REDUCTASE 1 or (1 of 1) PTHR12804//PTHR12804:SF2 - MICROSOMAL SIGNAL PEPTIDASE 23 KD SUBUNIT SPC22/23 // SUBFAMILY NOT NAMED	0.001656	0.048244	2.341714
evm.TU.AsparagusV1_01.3401.V1.1	(1 of 1) PTHR19297//PTHR19297:SF93 - GLYCOSYLTRANSFERASE 14 FAMILY MEMBER // CORE-2/I-BRANCHING BETA-1,6-N-ACETYLGLUCOSAMINYLTRANSFERASE FAMILY PROTEIN	0.001669	0.048244	0.229771

evm.TU.AsparagusV1_02.1063.V1.1	(1 of 1) PTHR34687:SF1 - CHAPERONE PROTEIN DNAJ-LIKE PROTEIN	0.001706	0.048244	0.049059
evm.TU.AsparagusV1_02.1212.V1.1	(1 of 2) PTHR31945:SF13 - TRANSCRIPTION FACTOR ICE1-RELATED	0.001665	0.048244	5.693744
evm.TU.AsparagusV1_02.16.V1.1	(1 of 1) K02941 - large subunit ribosomal protein LP0 (RP-LP0, RPLP0)	0.001702	0.048244	4.086533
evm.TU.AsparagusV1_02.1923.V1.1 or evm.TU.AsparagusV1_02.1924.V1.1	(1 of 3) 2.3.1.138 - Putrescine N-hydroxycinnamoyltransferase	0.001696	0.048244	67.55161
evm.TU.AsparagusV1_02.1983.V1.1	(1 of 1) PTHR24349:SF131 - CALCIUM-DEPENDENT PROTEIN KINASE 17-RELATED	0.001664	0.048244	0.287359
evm.TU.AsparagusV1_02.339.V1.1 or evm.TU.AsparagusV1_02.340.V1.1	(1 of 2) PTHR11732//PTHR11732:SF164 - ALDO/KETO REDUCTASE // ALDOSE REDUCTASE-RELATED or (1 of 3) PTHR21231:SF7 - GPN-LOOP GTPASE 3	0.001599	0.048244	1.085935
evm.TU.AsparagusV1_03.1368.V1.1	(1 of 4) PTHR31008:SF3 - COP1-INTERACTING PROTEIN-RELATED PROTEIN-RELATED	0.001711	0.048244	36.36306
evm.TU.AsparagusV1_03.1416.V1.1	(1 of 1) PTHR11895//PTHR11895:SF39 - AMIDASE // OUTER ENVELOPE PROTEIN 64, CHLOROPLASTIC	0.001602	0.048244	2.080212
evm.TU.AsparagusV1_03.2593.V1.1	(1 of 2) PTHR10693//PTHR10693:SF25 - RAS GTPASE-ACTIVATING PROTEIN-BINDING PROTEIN // SUBFAMILY NOT NAMED	0.0017	0.048244	31.20863
evm.TU.AsparagusV1_03.2666.V1.1	Missing annotation	0.001662	0.048244	0.073819
evm.TU.AsparagusV1_03.664.V1.1	(1 of 3) 2.5.1.32 - 15-cis-phytoene synthase / PSase	0.001619	0.048244	0.004148
evm.TU.AsparagusV1_03.755.V1.1	Missing annotation	0.001662	0.048244	14.14652
evm.TU.AsparagusV1_03.902.V1.1	(1 of 1) PTHR31901:SF2 - INDOLE-3-ACETIC ACID-AMIDO SYNTHETASE GH3.5-RELATED	0.001676	0.048244	0.161342

evm.TU.AsparagusV1_04.1197.V1.1	(1 of 2) PTHR31906:SF5 - PLASTID-LIPID-ASSOCIATED PROTEIN 3, CHLOROPLASTIC-RELATED	0.001557	0.048244	3.453906
evm.TU.AsparagusV1_04.2676.V1.1	(1 of 1) PTHR22792//PTHR22792:SF60 - LUPUS LA PROTEIN-RELATED // SUBFAMILY NOT NAMED	0.001695	0.048244	2.414574
evm.TU.AsparagusV1_04.680.V1.1	(1 of 3) K02639 - ferredoxin (petF)	0.001589	0.048244	3.298556
evm.TU.AsparagusV1_04.829.V1.1 or evm.TU.AsparagusV1_04.830.V1.1	(1 of 2) PTHR23201:SF16 - PROLINE-RICH PROTEIN 4	0.001559	0.048244	0.005877
evm.TU.AsparagusV1_05.186.V1.1	(1 of 66) PF01535//PF13041//PF14432 - PPR repeat (PPR) // PPR repeat family (PPR_2) // DYW family of nucleic acid deaminases (DYW_deaminase)	0.001649	0.048244	2.389424
evm.TU.AsparagusV1_05.2782.V1.1	(1 of 1) K02365 - separase (ESP1)	0.00171	0.048244	0.025915
evm.TU.AsparagusV1_05.2903.V1.1	(1 of 1) K12812 - ATP-dependent RNA helicase UAP56/SUB2 (UAP56, BAT1, SUB2)	0.001615	0.048244	1.866522
evm.TU.AsparagusV1_07.1123.V1.1	(1 of 2) PTHR11453:SF44 - BORON TRANSPORTER 1-RELATED	0.001694	0.048244	0.015788
evm.TU.AsparagusV1_07.1456.V1.1	(1 of 1) K14780 - ATP-dependent RNA helicase DHX37/DHR1 [EC:3.6.4.13] (DHX37, DHR1)	0.001577	0.048244	2.871513
evm.TU.AsparagusV1_07.2022.V1.1	(1 of 2) PTHR22884//PTHR22884:SF366 - SET DOMAIN PROTEINS // SUBFAMILY NOT NAMED	0.001673	0.048244	6.181425
evm.TU.AsparagusV1_07.575.V1.1	(1 of 3) K14293 - importin subunit beta-1 (KPNB1)	0.001655	0.048244	5.957327
evm.TU.AsparagusV1_08.1500.V1.1	(1 of 1) PTHR11850//PTHR11850:SF103 - HOMEOBOX PROTEIN TRANSCRIPTION FACTORS // SUBFAMILY NOT NAMED	0.001613	0.048244	0.072403
evm.TU.AsparagusV1_08.2320.V1.1	(1 of 1) PTHR10516//PTHR10516:SF214 - PEPTIDYL-PROLYL CIS-TRANS	0.001704	0.048244	53.3285

	ISOMERASE // PEPTIDYL-PROLYL CIS-TRANS ISOMERASE FKBP12			
evm.TU.AsparagusV1_08.2359.V1.1	(1 of 23) PF01357 - Pollen allergen (Pollen_allerg_1)	0.001567	0.048244	0.009204
evm.TU.AsparagusV1_08.2617.V1.1	(1 of 3) K02993 - small subunit ribosomal protein S7e (RP-S7e, RPS7)	0.001607	0.048244	4.480527
evm.TU.AsparagusV1_08.3072.V1.1	(1 of 2) PF13233 - Complex1_LYR-like (Complex1_LYR_2)	0.001639	0.048244	3.296453
evm.TU.AsparagusV1_08.344.V1.1	(1 of 2) KOG4308 - LRR-containing protein	0.00171	0.048244	4.410808
evm.TU.AsparagusV1_02.2250.V1.1	(1 of 1) 2.1.1.225 - tRNA:m(4)X modification enzyme	0.001721	0.048406	2.163572
evm.TU.AsparagusV1_02.1121.V1.1	(1 of 3) PTHR12300//PTHR12300:SF56 - HVA22-LIKE PROTEINS // SUBFAMILY NOT NAMED	0.001732	0.048506	0.095481
evm.TU.AsparagusV1_03.120.V1.1	(1 of 1) PTHR30620//PTHR30620:SF40 - PERIPLASMIC BETA-GLUCOSIDASE-RELATED // BETA-D-XYLOSIDASE 3-RELATED	0.001734	0.048506	0.364996
evm.TU.AsparagusV1_03.1874.V1.1	(1 of 2) PTHR11206//PTHR11206:SF80 - MULTIDRUG RESISTANCE PROTEIN // SUBFAMILY NOT NAMED	0.00173	0.048506	0.140649
evm.TU.AsparagusV1_02.258.V1.1 or evm.TU.AsparagusV1_02.259.V1.1	(1 of 1) PTHR10209//PTHR10209:SF199 - OXIDOREDUCTASE, 2OG-FE II OXYGENASE FAMILY PROTEIN // SUBFAMILY NOT NAMED or (1 of 1) PTHR10293//PTHR10293:SF45 - GLUTAREDOXIN-RELATED PROTEIN // MONOTHIOL GLUTAREDOXIN-S16, CHLOROPLASTIC	0.00174	0.048557	1.429618

evm.TU.AsparagusV1_01.3000.V1.1	(1 of 1) PTHR31769//PTHR31769:SF5 - FAMILY NOT NAMED // EXPRESSED PROTEIN	0.001745	0.04857	1.60092
evm.TU.AsparagusV1_06.1586.V1.1	(1 of 1) PTHR10288//PTHR10288:SF130 - KH DOMAIN CONTAINING RNA BINDING PROTEIN // SUBFAMILY NOT NAMED	0.001746	0.04857	3.350613
evm.TU.AsparagusV1_08.1200.V1.1	(1 of 1) K12883 - nuclear cap-binding protein subunit 2 (NCBP2, CBP20)	0.001754	0.048676	3.143846
evm.TU.AsparagusV1_02.378.V1.1	(1 of 1) PTHR11728//PTHR11728:SF13 - GLYCEROL-3-PHOSPHATE DEHYDROGENASE // GLYCEROL-3-PHOSPHATE DEHYDROGENASE [NAD(+)] 2, CHLOROPLASTIC	0.001802	0.048817	3.436696
evm.TU.AsparagusV1_02.769.V1.1	Missing annotation	0.001801	0.048817	0.053804
evm.TU.AsparagusV1_03.1647.V1.1	(1 of 2) K07203 - FKBP12-rapamycin complex-associated protein (FRAP)	0.001783	0.048817	2.788446
evm.TU.AsparagusV1_03.2021.V1.1	(1 of 7) K12741 - heterogeneous nuclear ribonucleoprotein A1/A3 (HNRNPA1_3)	0.001813	0.048817	3.230972
evm.TU.AsparagusV1_03.3127.V1.1	(1 of 8) K01507 - inorganic pyrophosphatase (ppa)	0.001791	0.048817	0.010494
evm.TU.AsparagusV1_04.2799.V1.1	(1 of 2) PTHR22835//PTHR22835:SF143 - ZINC FINGER FYVE DOMAIN CONTAINING PROTEIN // SUBFAMILY NOT NAMED	0.00179	0.048817	20.68986
evm.TU.AsparagusV1_04.380.V1.1	(1 of 1) K10807 - ribonucleoside-diphosphate reductase subunit M1 (RRM1)	0.001803	0.048817	6.79264
evm.TU.AsparagusV1_06.1478.V1.1	(1 of 7) PF03181 - BURP domain (BURP)	0.001772	0.048817	0.04363
evm.TU.AsparagusV1_07.2659.V1.1	Missing annotation	0.001791	0.048817	16.35446
evm.TU.AsparagusV1_08.3411.V1.1	(1 of 3) KOG2776 - Metallopeptidase	0.001788	0.048817	4.490079
evm.TU.AsparagusV1_10.1739.V1.1	(1 of 2) PTHR10257//PTHR10257:SF17 - SERINE/THREONINE PROTEIN	0.001792	0.048817	0.150842

	PHOSPHATASE 2A PP2A REGULATORY SUBUNIT B // SUBFAMILY NOT NAMED			
evm.TU.AsparagusV1_07.1669.V1.1	(1 of 10) K10406 - kinesin family member C2/C3 (KIFC2_3)	0.001819	0.048856	6.139387
evm.TU.AsparagusV1_08.1365.V1.1	(1 of 2) PTHR31558:SF1 - F7A19.6 PROTEIN-RELATED	0.001823	0.048856	0.00103
evm.TU.AsparagusV1_02.1190.V1.1	Missing annotation	0.001826	0.048865	0.01662
evm.TU.AsparagusV1_02.614.V1.1	(1 of 3) PTHR35753//PTHR35753:SF1 - FAMILY NOT NAMED // PROLINE-RICH FAMILY PROTEIN	0.001853	0.048865	2.640637
evm.TU.AsparagusV1_03.927.V1.1	(1 of 39) K00430 - peroxidase (E1.11.1.7)	0.001866	0.048865	0.721615
evm.TU.AsparagusV1_04.762.V1.1	Missing annotation	0.001852	0.048865	2.233318
evm.TU.AsparagusV1_06.1119.V1.1	(1 of 3) 5.3.99.9 - Neoxanthin synthase / NSY	0.001833	0.048865	0.058859
evm.TU.AsparagusV1_07.2105.V1.1	(1 of 477) PF01535 - PPR repeat (PPR)	0.001868	0.048865	7.828781
evm.TU.AsparagusV1_07.2750.V1.1	Missing annotation	0.001866	0.048865	0.038891
evm.TU.AsparagusV1_07.3432.V1.1	(1 of 1) PTHR22939//PTHR22939:SF76 - SERINE PROTEASE FAMILY S1C HTRA-RELATED	0.001861	0.048865	1.538376
evm.TU.AsparagusV1_07.528.V1.1	Missing annotation	0.001847	0.048865	7.201143
evm.TU.AsparagusV1_07.868.V1.1	(1 of 1) PTHR10759//PTHR10759:SF3 - 60S RIBOSOMAL PROTEIN L34 // SUBFAMILY NOT NAMED	0.001863	0.048865	0.590338
evm.TU.AsparagusV1_09.213.V1.1	(1 of 6) PTHR13832//PTHR13832:SF352 - PROTEIN PHOSPHATASE 2C // PROTEIN PHOSPHATASE 2C 59-RELATED	0.001841	0.048865	1.573059
evm.TU.AsparagusV1_01.2207.V1.1	(1 of 1) PTHR11735//PTHR11735:SF14 - O-SIALOGLYCOPROTEIN ENDOPEPTIDASE // TRNA N6-ADENOSINE THREONYLCARBAMOYLTRANSFERASE-RELATED	0.001892	0.049042	2.34978

evm.TU.AsparagusV1_01.3383.V1.1	(1 of 2) PTHR19241//PTHR19241:SF286 - ATP-BINDING CASSETTE TRANSPORTER // SUBFAMILY NOT NAMED	0.001892	0.049042	0.012283
evm.TU.AsparagusV1_01.3579.V1.1	(1 of 3) PTHR24012:SF318 - RNA-BINDING (RRM/RBD/RNP MOTIFS) FAMILY PROTEIN	0.001918	0.049042	13.31397
evm.TU.AsparagusV1_02.2062.V1.1	(1 of 2) PTHR11514//PTHR11514:SF57 - MYC // SUBFAMILY NOT NAMED	0.001891	0.049042	6.47704
evm.TU.AsparagusV1_02.567.V1.1	(1 of 4) PTHR10992//PTHR10992:SF869 - ALPHA/BETA HYDROLASE FOLD-CONTAINING PROTEIN // SUBFAMILY NOT NAMED	0.001919	0.049042	0.149713
evm.TU.AsparagusV1_06.1390.V1.1	Missing annotation	0.001907	0.049042	0.434291
evm.TU.AsparagusV1_06.881.V1.1 or evm.TU.AsparagusV1_06.882.V1.1	(1 of 41) PF14368 - Probable lipid transfer (LTP_2)	0.001922	0.049042	53.03969
evm.TU.AsparagusV1_07.121.V1.1 or evm.TU.AsparagusV1_07.122.V1.1	Missing annotation or (1 of 1) PF00228 - Bowman-Birk serine protease inhibitor family (Bowman-Birk_leg)	0.001921	0.049042	0.168377
evm.TU.AsparagusV1_07.1445.V1.1	(1 of 13) PF00407 - Pathogenesis-related protein Bet v I family (Bet_v_1)	0.001917	0.049042	3.091772
evm.TU.AsparagusV1_07.3543.V1.1	(1 of 1) K12587 - exosome complex component MTR3 (MTR3, EXOSC6)	0.00188	0.049042	7.563934
evm.TU.AsparagusV1_03.715.V1.1	(1 of 7) PF00069//PF00560//PF08263 - Protein kinase domain (Pkinase) // Leucine Rich Repeat (LRR_1) // Leucine rich repeat N-terminal domain (LRRNT_2)	0.001952	0.04967	3.801667
evm.TU.AsparagusV1_06.1093.V1.1	(1 of 3) PTHR10791:SF41 - BIDIRECTIONAL SUGAR TRANSPORTER SWEET6-RELATED	0.00196	0.04967	0.00948
evm.TU.AsparagusV1_08.2130.V1.1	(1 of 16) 2.4.1.324 - 7-deoxyloganetin glucosyltransferase / UGT85A24	0.001971	0.049705	5.299289
evm.TU.AsparagusV1_02.608.V1.1	(1 of 2) K17081 - prohibitin 2 (PHB2)	0.001979	0.049768	5.780689

evm.TU.AsparagusV1_02.954.V1.1	(1 of 2) 4.1.1.36 - Phosphopantothenoylcysteine decarboxylase / N-((R)-4'-phosphopantothenoyl)-L-cysteine carboxy-lyase	0.001978	0.049768	4.585321
evm.TU.AsparagusV1_10.1726.V1.1	(1 of 4) K02943 - large subunit ribosomal protein LP2 (RP-LP2, RPLP2)	0.001982	0.049799	3.82058
evm.TU.AsparagusV1_02.856.V1.1	Missing annotation	0.001992	0.049839	0.03376
evm.TU.AsparagusV1_07.1097.V1.1	(1 of 1) K13111 - WD40 repeat-containing protein SMU1 (SMU1)	0.00199	0.049839	4.149978

Table 9. Stock concentrations of antibiotics used in transformation

Antibiotic (abbreviation)	Stock concentration (mg/mL)
Hygromycin (Hyg)	100
Timentin (T)	200
Acetosyringone (AS)	100
Spectinomycin (Spec)	50
Claforan (CLA)	300

Table 10. Media recipes

Ingredients	Callus Induction Media (CIM)	Shoot Induction Media (SIM)	Shoot Elongation Media (SEM)
MS Basal Salt Mixture	17.22 g	17.22 g	17.22 g
BAP (0.5 mg/mL stock)	4 mL	2 mL	0.25 mL
NAA (0.1 mg/mL stock)	0.5 mL	0.5 mL	0.5 mL
pH	5.7	5.7	5.7
Gellan gum	1.5 g	1.5 g	1.5 g

Ingredients	1/2 MS
1/2 MS salt	1.08 g
Sucrose	10 g
MS vitamins	0.5 mL
L-glutamine	0.01 g
pH	5.8
MES	0.125 g
pH	5.8
Gellan gum	2 g